Phytochemical Investigation on the Seeds of *Brucea antidysenterica*

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

By
Haregewine Tadesse
July, 2005

Dedicatd to*:* My lovely family

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Declaration

I declare that this thesis is my original work and has not been presented for any degree in this or other University and that all sources of materials used for this thesis have been duly acknowledged.

Name: Haregewine Tadesse

Signature: ___________________

This thesis has been submitted for examination with approval as a University advisor.

Name: Prof. Ermias Dagne

Signature: ___________________

Place and date of submission: School of Graduate Studies of Addis Ababa University

July, 2005

Addis Ababa University

School of Graduate Studies

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Department of Chemistry

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Finally, I give all the glory, the honor and praise to my Lord and Savior Jesus Christ!

Abstract

Brucea antidysenterica J. F. Mill. (Simaroubaceae) is a well known shrub from the bark of the plant many quassinoids including the antileukemic compounds bruceantin, which was in phase II clinical trial by the National Cancer Institute (USA).

In this work it was possible to isolate four compounds from the seeds: two glycosidic quassinoids (BA5 and BA6), one alkaloid (BA2) and one flavonoid (BA7B). Their structures were determined by spectral measurements and comparison with the previous work.

1. Introduction

1.1. Ecological distribution of Simaroubaceae family

The family Simaroubaceae comprises medium sized family of about 25 genera and 120 species which are mainly small trees or shrubs, distributed widely in tropical America and West Africa. Some genera of this family include *Brucea* J.F. Mill (1779), *Harrisonia* R. Br. ex A. Juss. (1825) and *Kirkia* Oliv. (1868) (Hedberg et al., 1989). A typical characteristic of these plants is the presence of bitter substances.

Brucea is a genus of about 10 species occurring in Africa, Asia and Australia (Table 1). In Ethiopia two species are found and *B. antidysenterica* is the most well known of these.

Table 1: *Brucea* species and their occurrence.

Species of the genus *Brucea*	**Occurence**	**Reference**
B. antidysenterica	Ethiopia, Asia, Angola, Cameroon, Congo, Sudan, Malawi, Tanzania	Hedberg et al., 1989
B. erythraeae	Eritrea	Brian et al., 2000
B. tenuifolla	Uganda and Tanzania	Hedberg et al., 1989
B. macrocarpa	Kenya	‘’
B. salutaris	Guinea	‘’
B. ferruginea	Ethiopia	‘’
B. sumatrana	Asia	Keng et al., 1968
B. amarissima	“”	‘’
B. javanica	‘’	‘’
B. mollis	‘’	‘’

B. antidysenterica

It is shrub or small tree from 10 to 15 m high (Fig.1), with smooth bark of grey to pale brown color with distribution in tropical Africa: Guinea, Nigeria, Ethiopia, Cameroon, Congo (DRC), Burundi, Sudan, Angola, Zambia and Malawi. The young stems are covered with ferruginous pubescence, leaves are 10-64 cm long and seed 8-9 mm long and 5-6 mm wide. Up-land (1400 to 2800 m) high evergreen forest and forest margins are very suitable area for the plant (Brian et al., 2000).

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Fig. 1: *B. antidysenterica* (Picture by Prof. E. Dagne)

1.2. Ethnobotany of *Brucea* species

The root barks of the tree known locally as *Waginos* (in Geez') or *Yedega Abalo* (in Amharic) were used by people living in northern Ethiopia for many centuries for treating dysentery. The British traveler James Bruce who stayed in Ethiopia from 1769 to 1771 was attacked by dysentery when he was about to leave Ethiopia. When observing that he would not be able to make it to Europe traveling through the hot landmass of Sudan and Egypt, the chief of Ganjar informed him to take a local drug known as *Waginos*. The root barks of this plant were cleaned, dried in the sun, and ground into powder. Bruce was then made to take two spoonfuls of the powder with camel's milk. After the sixth or seventh day Bruce regained his health and was able to continue his journey. On his way back, he took some of the fruits of *Waginos* which he delivered to the botanist Daniel Solander at the British Museum, who noting that it represented a taxon not known then in Europe had it planted in several British gardens. The plant was later named *Brucea antidysenterica* J. F. Miller in honor of James Bruce and with the specific epithet indicating the medicinal property of the plant.

The fruits, leaves and roots of *B. antidysenterica* are mixed with small amount of water and applied to treat wound. Afterwards cleaning the body with solution made of the powdery leaves of *Rumex nervosus* (Enbuacho- in Amharic) (Mesfin Tadesse, 1986).

According to Keng et al. (1968), *B. sumatrana, B. amarissima , B. antidysenterica,* and *B. javanica* are species found in Asia and known by local names *ko-sam, ya-tan-tzu, k'u-shen-tzu,* and *lao-ya-tan* respectively. They were used as herbal remedies against human amoebiasis.

1.3. Biological activities of *Brucea* species

1.3.1. Anti-inflammatory

Bruceantin (**2**) and analogues compounds are triterpenes of the quassinoid type isolated from the bark of the Ethiopian tree *B. antidysenterica.* They are capable of inducing an array of biological responses including anti-inflammatory effect with murine models (Kupchan et al., 1973).

1.3.2. Anti-amoebiasis

Bruceantin (**2**) shows marked amoebicidal activity *in vitro* in the colony assay. At concentration of 0.2 μg/ml and above, all of the parasites *Entamoeba histolytica* (the dysentery amoeba) are killed. At 0.1 μg/ml, few parasites survive at 48 h, and all of the parasites are killed after 72 h to 0.1 μg/ml (Frances et al., 1982).

1.3.3. Anti-malarial Activity

B. javanica fruits used traditionally in Chinese medicine to treat malaria are taken in the form of aqueous infusions. *In vitro* antiplasmodial tests of the fruits utilizing *Plasmodium falciparum* showed significant antimalarial activity with $IC_{50} = 0.006$ μg/ml (Anderson et al., 1990). In addition bruceantin, a constituent of the fruits from *B. javanica* is active against 3 strains of *P. falciparum* in vitro; all 3 strains are chloroquine resistant (Guru et al., 1983).

1.3.4. Anti-cancer Activity

The alcoholic extract of *B. antidysenterica* showed significant inhibitory activity *in vitro a*gainst cells derived from human carcinoma of the nasopharynx (KB), Walker 256 intramuscular carcinosarcoma in the rat, and P-388 lymphocytic leukemia in the mouse (PS) (Kupchan et al., 1975).

Several of the analogues compounds of bruceantin (**2**) exhibited significant anti-cancer activities with different models. According to Muriel et al. (2004), the most responsible mechanism at the molecular level has been attributed to inhibition of protein synthesis *via* interference at the peptidysl transferase site, thus preventing peptide bond formation. Bruceantin was evaluated in three separate Phase I Clinical trials in patients with various types of solid tumors and in two separated Phase II trials including adult patients with metastatic breast cancer and malignant myeloma. Due to the inadequate quantities of the compound (Website 1) and no objective tumor regressions observed, the clinical trials were terminated. In the recent studies, the effect of bruceantin (**2**) on multiple myeloma was tested and showed the following results. At lower doses (10 ng/ml for 24 h) tumor growth was completely inhibited but at higher doses loss of body weight or lethality were observed.

1.3.5. Anti-tuberculosis activity

In vitro evaluation of anti-tuberculosis activity was conducted for different quassinoids such as dehydrobruceantin (**5**). Though the activities were very low (0-19%), **5** showed potent anti-tuberculosis activity (Narihiko et al., 1997).

1.4. Review of the Chemistry of *Brucea* species

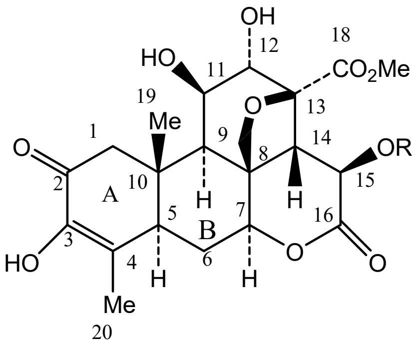
Quassinoids are bitter-tasting terpenoid compounds, which are characteristic of the Simaroubaceae family. The structures of these compounds isolated from *Brucea* species are deduced mainly based on chemical transformations and spectroscopic techniques such as IR, UV, MS, and 1D NMR.

Based on literature review the structures of 74 compounds which have been obtained form the genus *Brucea* are shown below. Brusatol (**1)** was most likely the first compound that was isolated from *Brucea* species i.e. *B. sumatrana* by Keng et al. (1968). Interest in the genus arose as a result of the discovery of bruceantin (**2**) and its analogs from the Ethiopian plant *B. antidysenterica* by the research group of Kupchan, who isolated and characterized about ten compounds. Structure activity relationship studies showed that quassinoids which possess the α,β-unsaturated ketol group at position 1 and 2 such as bruceantin (**2**), display antineoplastic activity in the murine lymphocytic leukemia P-388 system (Kupchan et al., 1973 and 1975).

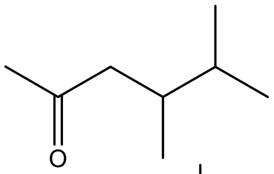
Kupchan and co-workers several research groups in the USA, China and Japan embarked on the study of other *Brucea* species in particular *B. javanica, B. amarisima, B. sumatrana* and *B. mollis.* The major types of compounds isolated todate are classified into four main groups as shown in the next pages.

I. Non glycosidic quassinoids

A. Brusatol, Bruceantin and their analogs



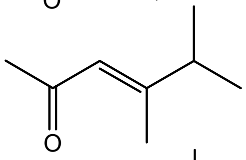
R=



Brusatol

1

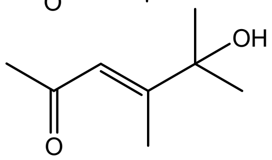
R=



Bruceantin

2

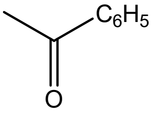
R=



Bruceantinol

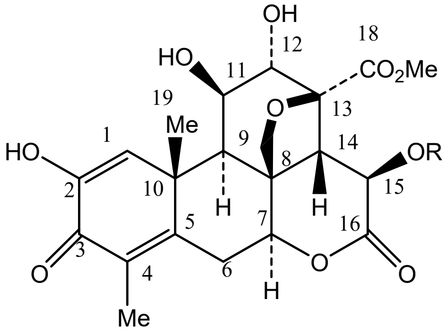
3

R=

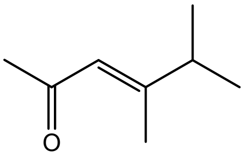


Bruceantarin

4



R=



Dehydrobruceantin

5

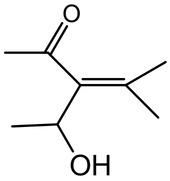
R= COC_6H_5 Dehydrobruceantarin

6

R= COCH3 Dehydrobrucein B

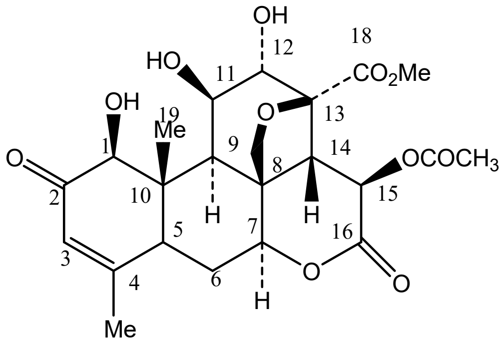
7

R=



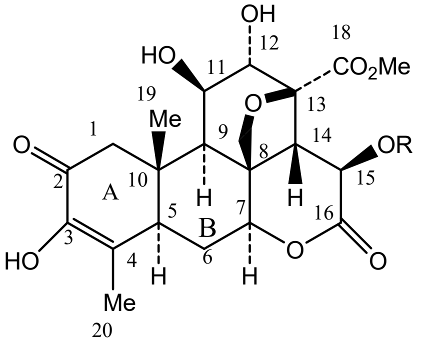
Dehydrobruceantol

8

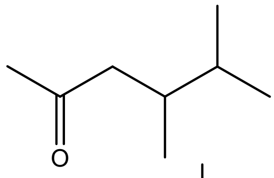


Isobruceine

9



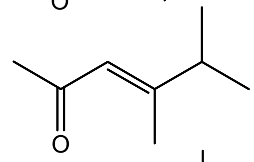
R=



Brusatol

1

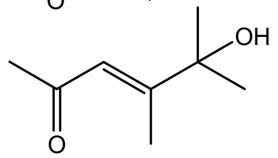
R=



Bruceantin

2

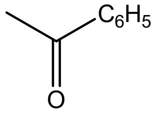
R=



Bruceantinol

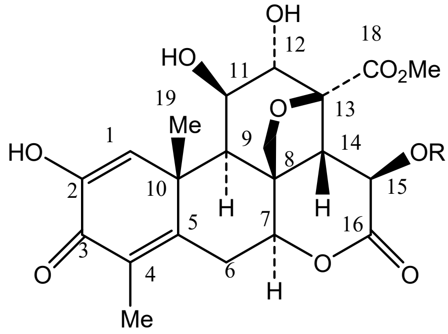
3

R=

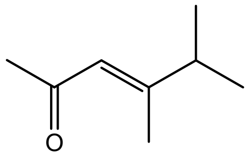


Bruceantarin

4



R=



Dehydrobruceantin

5

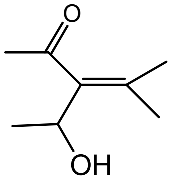
R= COC_6H_5 Dehydrobruceantarin

6

R= COCH3 Dehydrobrucein B

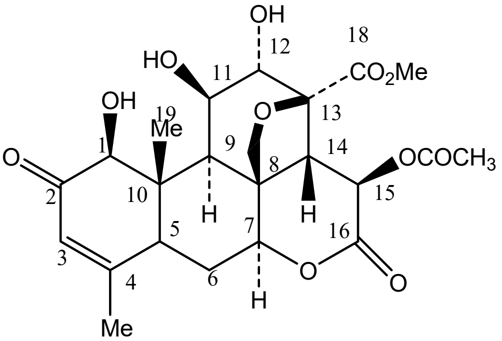
7

R=



Dehydrobruceantol

8

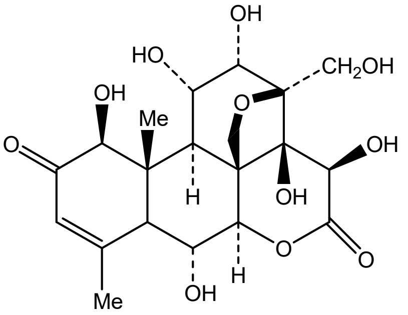


Isobruceine

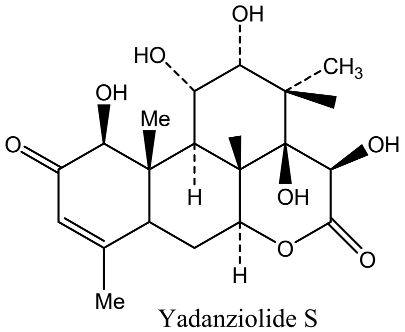
9

B. Yadanziolides

These are quassinoids in which there is no glucopyranosyl moiety.



Yadanziolide B
10

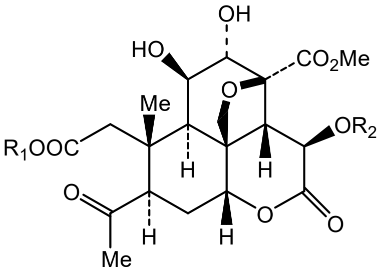


Yadanziolide S
11

Note: Due to the limitations of reference materials the structures of yadanziolide A and yadanziolides C-R are not given here.

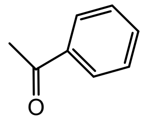
C. Bruceanic acids

These type of quassinoids contain acidic functional group.



R_1 = H,

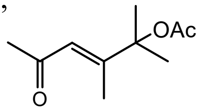
R_2 =



Bruceanic acid B
14

R_1 = H,

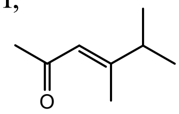
R_2 =



Bruceanic acid C
15

R_1 = H,

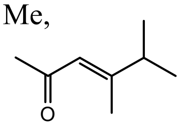
R_2 =



Bruceanic acid A
12

R_1 = Me,

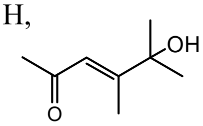
R_2 =



Bruceanic acid A methyl ester
13

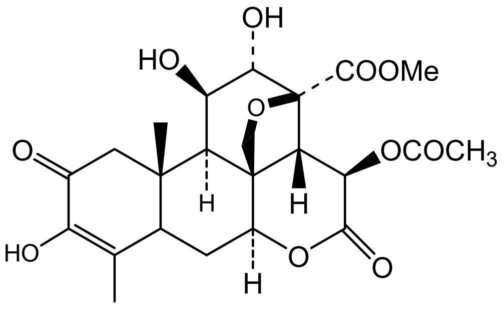
R_1 = H,

R_2 =



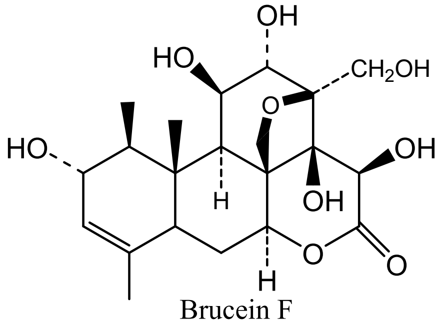
Bruceanic acid D
16

D. Bruceins



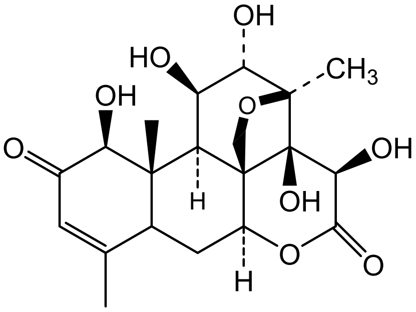
Brucein B

17



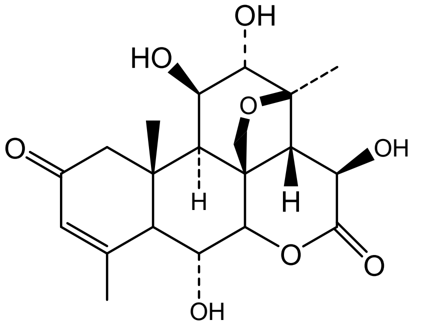
Brucein F

20



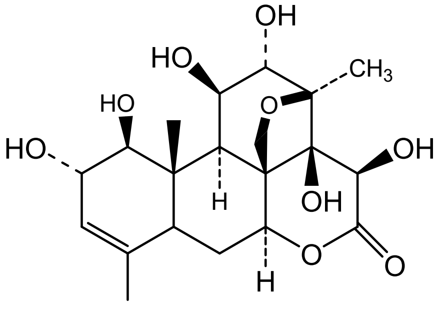
Brucein D

18



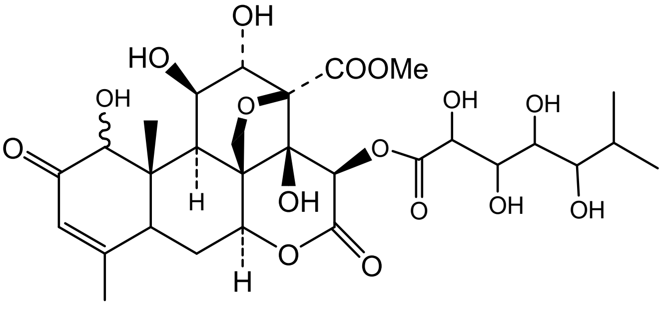
Brucein G

21



Brucein E

19

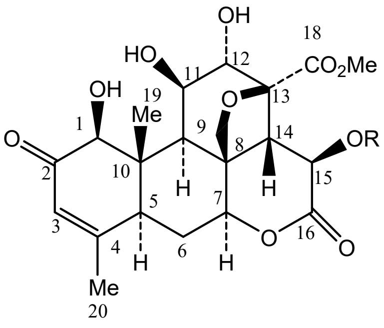


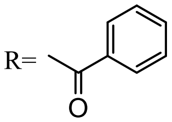
Brucein Q

22

Note: The structures of brucein A, C and bruceins H-P are not given here.

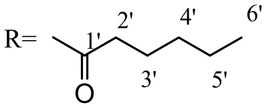
D. Bruceanols





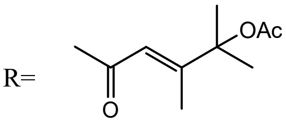
Bruceanol A

23



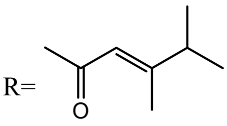
Bruceanol B

24



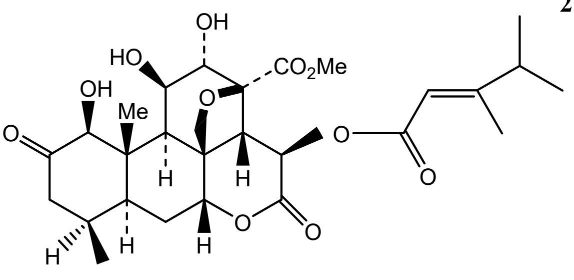
Bruceanol C

25



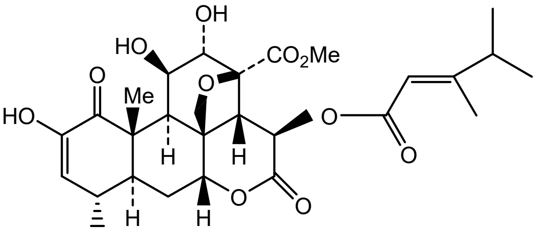
Bruceanol D

26



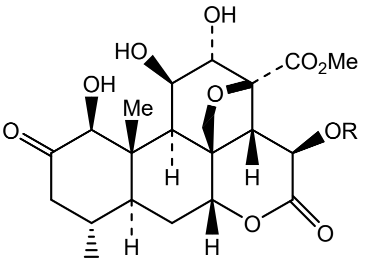
Bruceanol E

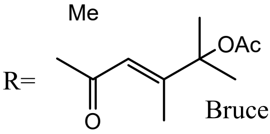
27



Bruceanol F

28





Bruceanol G

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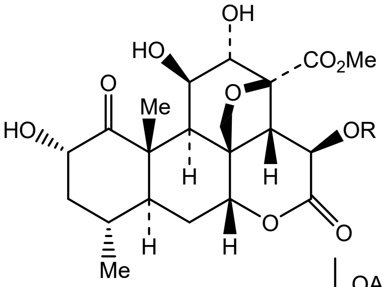
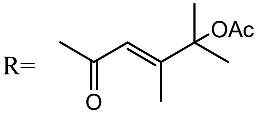
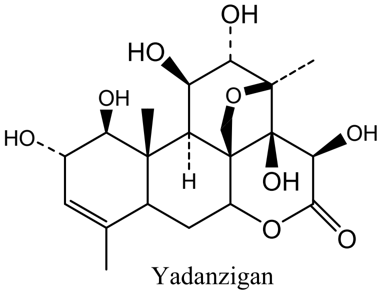




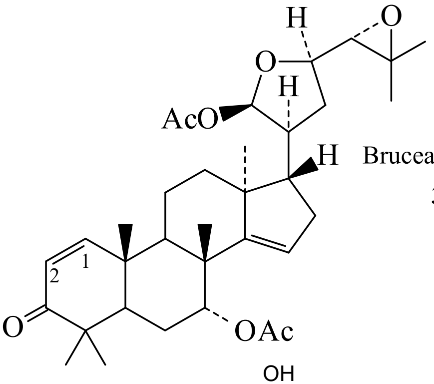
Bruceanol H

30

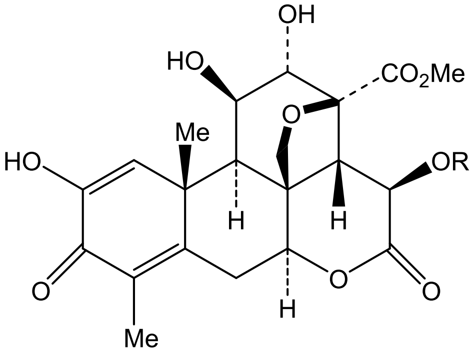
F. Miscellaneous, *Brucea* quassinoids and a triterpene

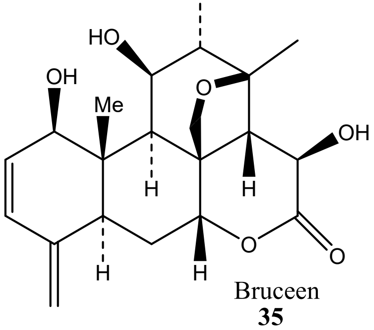


Yadanzigan
31



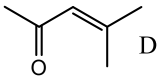
Brucea javanin A
34





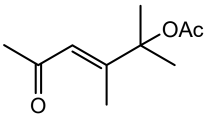
Bruceen
35

R=



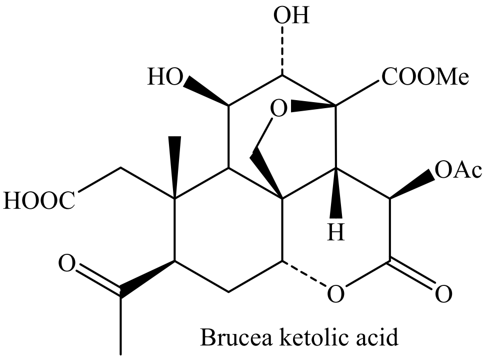
Dehydrobrusatol
32

R=



Dehydrobruceantinol

33



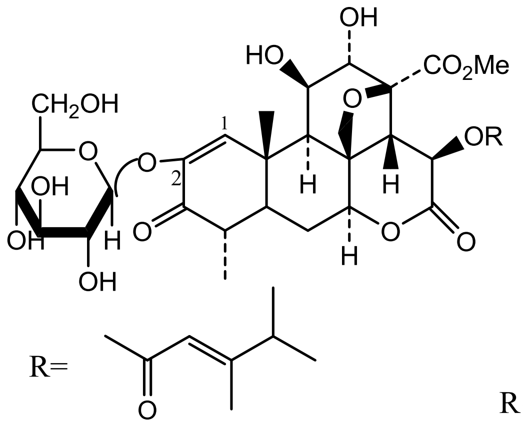
Brucea ketolic acid

36

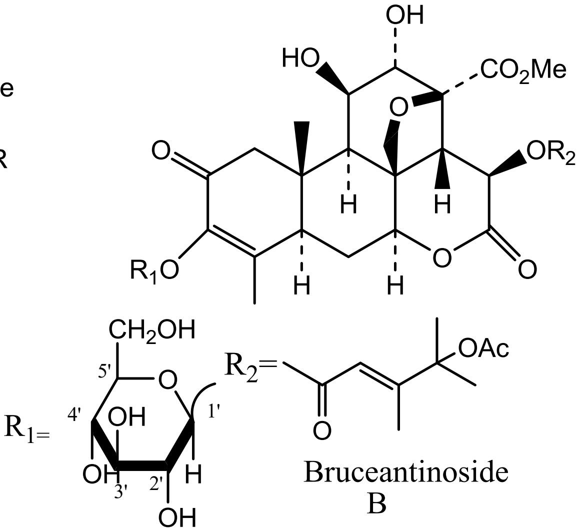
II. Glycosidic quassinoids

The common position of glycosylation observed for the quassinoids were C-1, C-2, C -3 of the compounds.

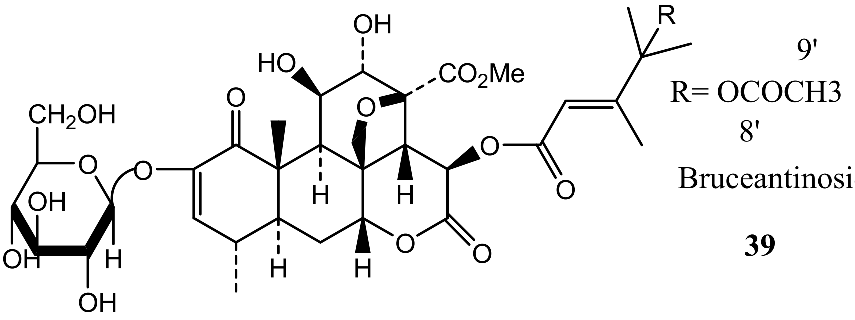
A. Bruceantinosides



Bruceantinoside A
37



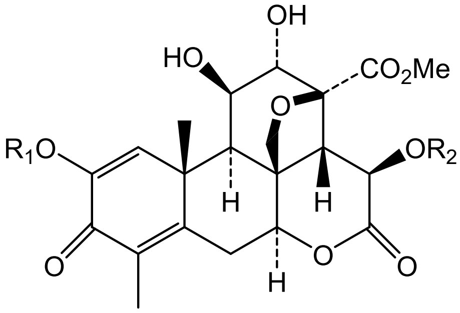
Bruceantinoside B
38

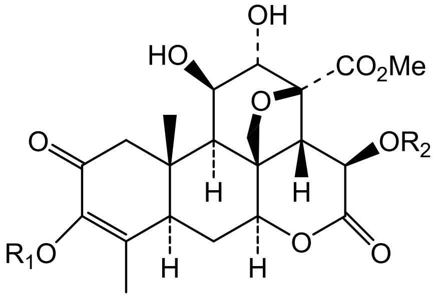


Bruceantinoside C

39

B. Bruceosides



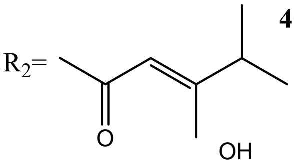


R_1 = *β*-Glc

Bruceoside A

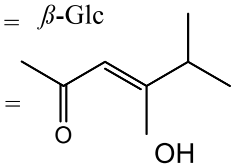
40

R_2=



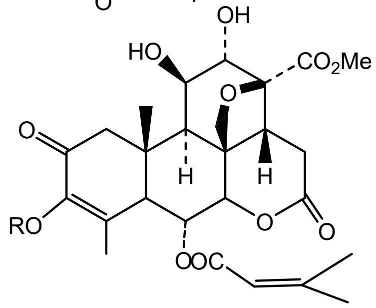
R_1 = *β*-Glc

R_2 =



Bruceoside B

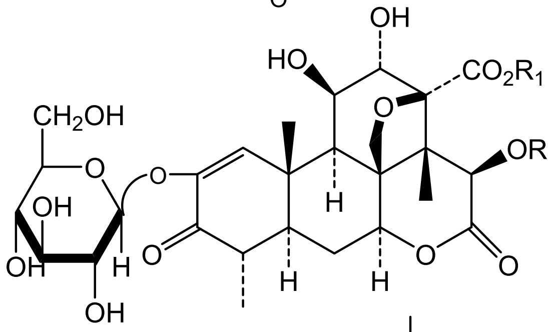
41



R=*β*-Glc

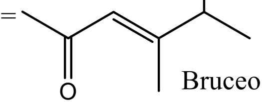
Bruceoside C

42



R_1=H

R_2=

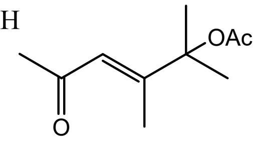


Bruceoside D

43

R_1=H

R_2=

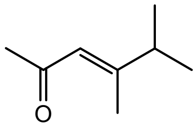


Bruceoside F

45

R_1=H

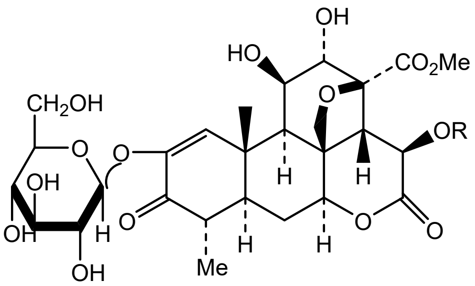
R_2=



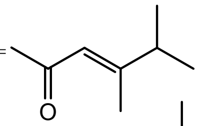
Bruceoside E

44

C. Yadanziosides

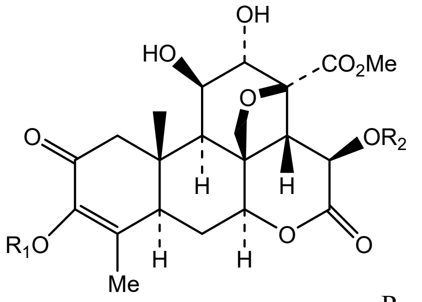


R=



Yadenzioside A

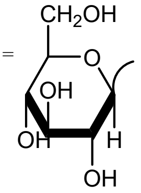
46



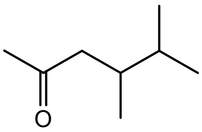
Yadenzioside B

47

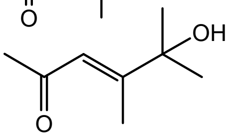
R_{1}=



R_2 =



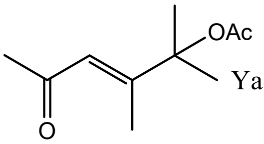
R=



Yadenzioside C

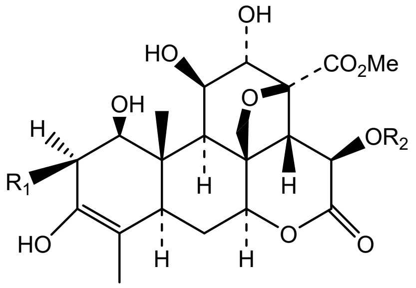
48

R=



Yadenzioside G

51



R_1= *O*-*ß*-Glc

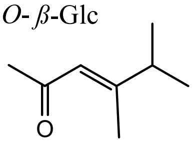
R_2 = $COCH_3$

Yadenzioside D

49

R_1= *O*-*ß*-Glc

R_2 =

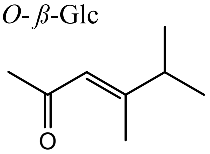


Yadenzioside E

50

R_1= *O*-*ß*-Glc

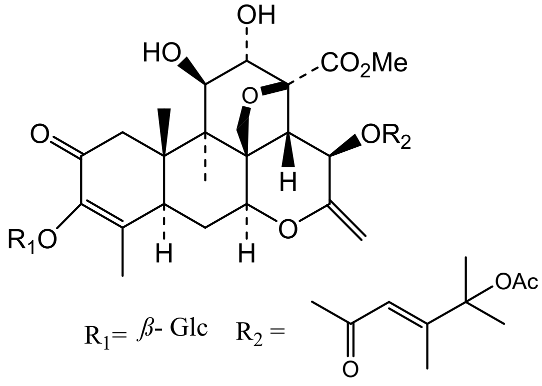
R_2 =



Yadenzioside H

56

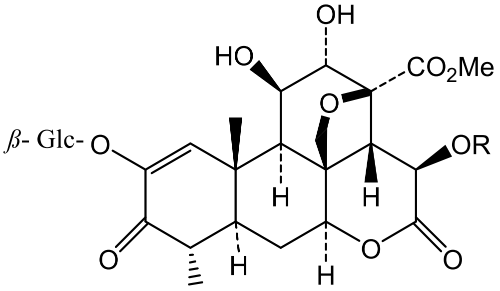
Note: The structures of yadanziosides F, I, J and L are not given.



R_1= *β*- Glc R_2 =

Yadanzioside K

57

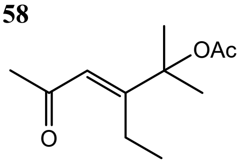


R = $OCOC_6H_5$

Yadanzioside M

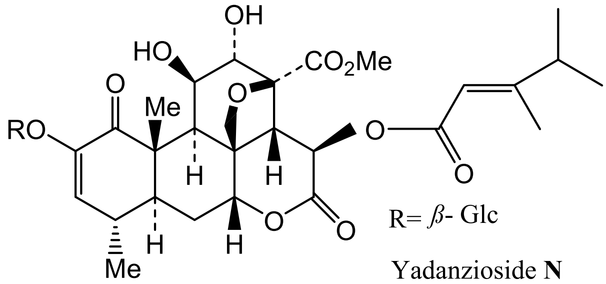
58

R =



Yadanzioside O

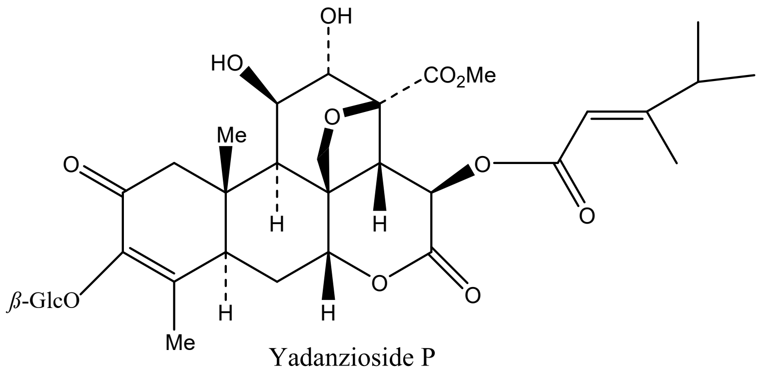
60



R= *β*- Glc

Yadanzioside **N**

59



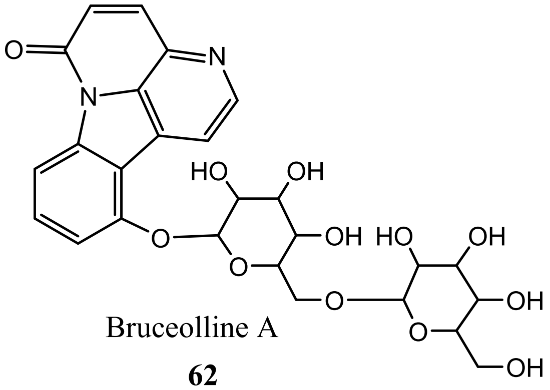
Yadanzioside P

61

III. Glycosidic alkaloids

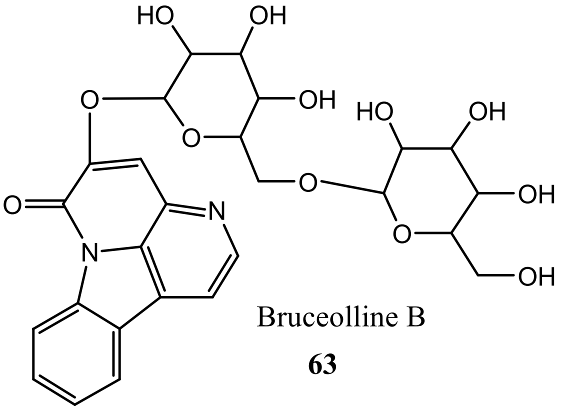
Bruceollines

In the case of glycosidic bruceollines, glycosilation occurs at different positions on the compounds.



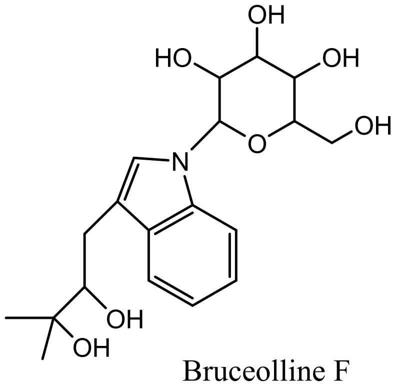
Bruceolline A

62



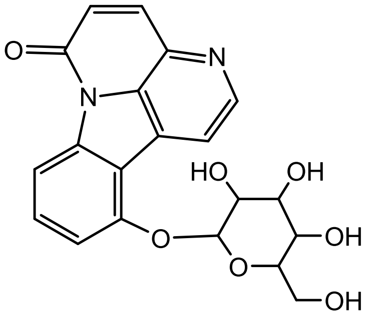
Bruceolline B

63



Bruceolline F

64

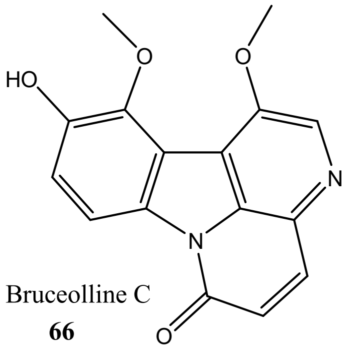


Bruceolline G

65

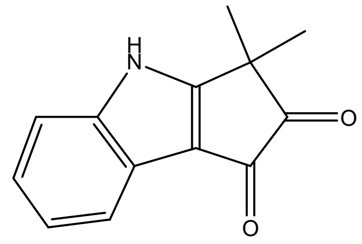
IV. Non glycosidic alkaloids

Bruceollines



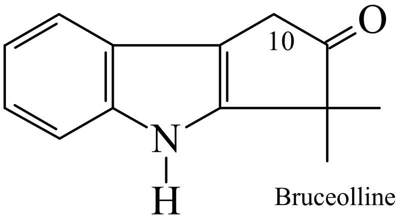
Bruceolline C

66



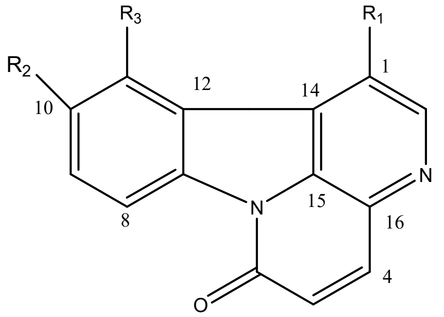
Bruceolline E

68



Bruceolline D

67

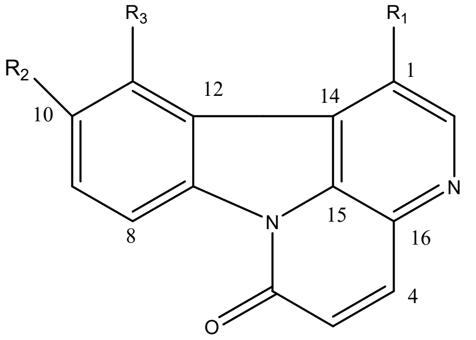


R_1= H, R_2 = OH 11-hydroxycanthin-6-one **70**

R_1= R_2 = H canthin-6-one **69**

R_1= R_2 = OMe 1, 11-dimethoxycanthin-6-one

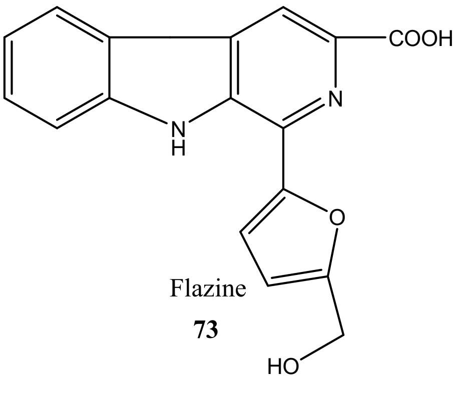
71



R_1 = OH, R_2 =H, R_3 =OMe

1-hydroxy-11-methoxycanthin-6-one

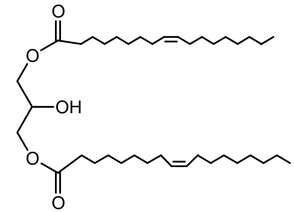
72



Flazine

73

V. Miscellaneous



74

Table 2 summarizes the sources of the above structures.

Table 2: Summary of compounds isolated from the genus *Brucea*

Compound	**Structure No.**	**Source**	**Reference**
Non glycosidic quassinoids			
Brusatol	**1**	*B. sumatrana*	Keng et al., 1968
Bruceantin	**2**	*B. antidysenterica*	Kupchan et al., 1975
Bruceantinol	**3**	‘ ’	‘ ’
Bruceantarin	**4**	‘ ’	‘ ’
Dehydrobruceantin	**5**	‘ ’	‘ ’
Dehydrobruceantarin	**6**	‘ ’	‘ ’
Dehydrobrucein	**7**	‘ ’	‘ ’
Dehydrobruceantol	**8**	‘ ’	‘ ’
Isobruceine	**9**	‘ ’	‘ ’
Yadanizolide B	**10**	*B. javanica*	DNP*
Yadanziolide S	**11**	‘ ’	Bao-Ning et al., 2002
Bruceanic acid A	**12**	*B. antidysenterica*	Toyota et al., 1990
Methyl ester of **12**	**13**	‘ ’	‘ ’
Bruceanic acid B	**14**	‘ ’	‘ ’
Bruceanic acid C	**15**	‘ ’	‘ ’
Bruceanic acid D	**16**	‘ ’	‘ ’
Bruceins B	**17**	*B. amarissima*	DNP
Bruceins D	**18**	*B. javanica*	‘ ’
Bruceins E	**19**	*B. amarissima*	‘ ’
Bruceins F	**20**	‘ ’	‘ ’
Bruceins G	**21**	*B. sumatrana*	‘ ’
Bruceins Q	**22**	‘ ’	‘ ’
Bruceanols A	**23**	*B. antidysenterica*	Masayoshi et al., 1985
Bruceanols B	**24**	‘ ’	‘ ’
Bruceanols C	**25**	‘ ’	Narihiko et al., 1988
Bruceanols D	**26**	‘ ’	Imamura et al., 1993
Bruceanols E	**27**	‘ ’	‘ ’
Bruceanols F	**28**	‘ ’	‘ ’
Bruceanols G	**29**	‘ ’	Imamura et al., 1995
Bruceanols H	**30**	‘ ’	DNP
Yadanzigan	**31**	*B. javanica*	‘ ’
Dehydrobrusatol	**32**	‘ ’	Toshiro et al., 1985
Dehydrobruceantinol	**33**	‘ ’	‘ ’
Brucea javanin A	**34**	*B. javanica*	DNP
Bruceen	**35**	‘ ’	‘ ’
Brucea ketolic acid	**36**	‘ ’	‘ ’

Table 2: contd...

Compound	**Structure No.**	**Source**	**Reference**
Glycosidic quassinoids			
Brucantinoside A	**37**	*B. antidysenterica*	Masayoshi et al., 1981
Brucantinoside B	**38**	‘’	‘’
Brucantinoside C	**39**	‘’	Narihiko et al., 1986
Bruceoside A	**40**	*B. javanica*	Lee et al,. 1979
Bruceoside B	**41**	‘’	DNP
Bruceoside C	**42**	‘’	‘’
Bruceoside D	**43**	‘’	‘’
Bruceoside E	**44**	‘’	‘’
Bruceoside F	**45**	‘’	‘’
Yadanzioside A	**46**	‘’	Toshiro et al., 1985
Yadanzioside B	**47**	‘’	‘’
Yadanzioside C	**48**	‘’	‘’
Yadanzioside D	**49**	‘’	‘’
Yadanzioside E	**50**	‘’	‘’
Yadanzioside G	**51**	*B. antidysenterica and B. javanica*	‘’
Yadanzioside H	**56**	*B. javanica*	DNP
Yadanzioside K	**57**	‘’	Toshiro et al., 1986
Yadanzioside M	**58**	‘’	‘’
Yadanzioside N	**59**	*B. antidysenterica and B. javanica*	‘’ ‘’
Yadanzioside O	**60**	*B. javanica*	‘’
Yadanzioside P	**61**	*B. antidysenterica and B. javanica*	Masayoshi et al., 1989
Glycosidic alkaloids			
Bruceolline A	**62**	*B. mollis*	Yishan et al., 1994
Bruceolline B	**63**	‘’	‘’
Bruceolline F	**64**	‘’	‘’
Bruceolline G	**65**	‘’	Yishan et al., 1995

Table 2: contd...

Compound	**Structure No.**	**Source**	**Reference**
Non glycosidic alkaloids			
Bruceolline C	**66**	*B. mollis*	Yishan et al., 1995
Bruceolline D	**67**	‘’	Yishan et al., 1994
Bruceolline E	**68**	‘’	‘’
Canthin-6-one	**69**	*B. antidysenterica*	Narihiko et al., 1986
11-hydroxycanthin-6-one	**70**	‘’	‘’
1,11-dimethoxycanthin-6-one	**71**	‘’	‘’
1-hydroxy-11-methoxy canthin-6-one	**72**	*B. mollis*	Yishan et al., 1995
Flazin	**73**	*B. javanica*	Bao-Ning et al., 2002
Others			
Compound 74	**74**	‘’	‘’

*DNP: Dictionary of Natural Products, 2005.

1.4. Objective of the study

The objective of this work is to elucidate structures of compounds isolated from the seeds of *B. antidysenterica*.

2. Results and Discussion

The pulverized ground seeds (1.5 kg) of *B. antidysenterica* was soaked in 90% aqueous ethanol at room temperature for about two days, filtered and the filterate concentrated using rotavapor to give a mixture of oil and brown tar. After defatting the oil by partitions the extract using petrol then diluting it to 70% aqueous ethanol, partition with dichloromethane gave the extract (89-53A). Dilution of the 70% aqueous ethanol layer to 50% and another partition with ethyl acetate yielded 89-53C. The TLC of the two extracts showed the presence of at least eight spots for 89-53A and about seven major spots for 89-53C when observed under 254 nm UV-lamp.

In this work it has been possible to isolate four compounds which exhibited R_f values (0.45) BA2, (0.6) BA5, (0.5) BA6 and (0.6) BA7B using different chromatographic techniques (Experimental Section for details).

The characterization of BA2 was done based on UV, MS, IR, 400 MHz 1 and 2D NMR spectra and comparing with the literature values. Partial characterization of BA7B, BA5 and BA6 were also done by using similar techniques.

2.1. Characterization of BA2

BA2 was obtained as a light yellow solid and recrystallized from methanol to give needle crystals. The mass data (Table 3) of this compound showed a molecular ion $[M]^+$ peak (HR-ESIMS) at m/z 199.252 corresponding to the molecular formula $C_{13}H_{13}NO$. Similarities were observed between the IR and MS spectra data of BA2 with a compound firstly synthesized (Fig. 3) (Ernest et al., 1986) and isolated later from *Pambrus missionis* (Vijaya et al., 1993).

The IR (KBr) spectrum of the compound showed a band at ν_{max} 3275 cm^{-1} due to N-H stretching. In addition to that the presence of a conjugated carbonyl group also attested by the band appearing at ν_{max} 1643 cm^{-1} (Table 3).

Table 3: Comparison of IR and MS of compound **52** with literature values.

Parameters (spectroscopic)	**BA2**	**Compound 52** (Ernest et al., 1986)	**Compound 52** (Vijaya et al., 1993)
IR (cm^{-1})	3275, 1643	3445, 1640	3270, 1640
MS $[M]^+$, m/z	199	199	199

Inspection of the ^1H NMR spectrum (Table 4) of the compound showed the presence of aromatic multiplet protons between δ_H 7.15 - 7.4 and at δ_H 8.47. A broad singlet peak was also observed at δ_H 8.9 due to N-H exchangeable proton. This was confirmed by observing another peak at δ_H 4.8 due to the formation of HOD after the addition of a drop of deutrated water. The proton appearing as a doublet at δ_H 7.75 as the result of coupling with NH proton suggested it to be H-2. One vinylic singlet proton was also observed at δ_H 6.6. Furthermore the two sharp singlets at δ_H 1.98 and 2.20 were assigned for two methyl carbons.

In the ^{13}C and DEPT-135 spectra (Table 4), five quaternary carbons were observed at δ_C 188.1 (carbonyl carbon), and four other unsaturated carbons at δ_C 153.1, 136.8, 126.3 and 120.3.

In the 2D NMR spectra of the compound the following correlations were observed. The HSQC spectrum showed correlation of the two methyl singlet protons at δ_H 1.98 and 2.20 with the two carbons at δ_C 28.1 and 21.2 respectively. The vinylic proton at δ_H 6.6 correlated with C-2' (δ_C 122.9) while the aromatic multiplet protons between δ_H 7.15-7.4 and 8.47 showed correlations with the carbons at δ_C 111.7, 122.8, 123.0 and 123.9. In addition one doublet proton appearing at δ_H 7.75 is attached to C-2 (δ_C 131.0) based on the observed correlation.

The following partial structures could be suggested based on the observed correlations from the HMBC spectrum (Table 4).

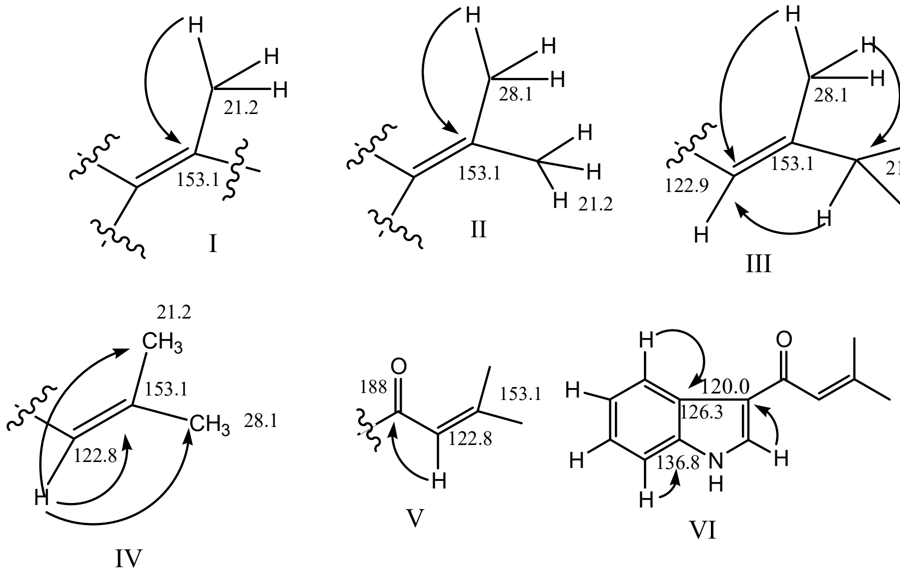


Fig. 2: Selected HMBC correlations of BA2 and partial structures.

Table 4: ^{1}H, ^{13}C, and HMBC (^{1}H →^{13}C) NMR data of compound **52** in $CDCl_3$.

C.No.	δ$_C$	δ$_H$	HMBC ^{1}H → ^{13}C
2	131.0 (*d*)	7.75 (*d*)	-
3	120.0 (s)	-	H-4, H-2
3a	126.3 (*s*)	-	H-2, H-4, H-5
4	122.8 (*d*)	8.47 (*m*)	-
5	123.0 (*d*)	7.15-7.4 (*m*)	-
6	123.9 (*d*)	‘’	-
7	111.7 (*d*)	‘’	-
7a	136.8 (*s*)	-	H-2, H-7
1’	188.1 (s)	-	H-2’
2’	122.9 (*d*)	6.6 (*s*)	H-4’, H-5’
3’	153.1 (*s*)	-	H-4’, H-5’
4’	28.1 (*q*)	1.98 (*s*)	H-5’, H-2’
5’	21.2 (*q*)	2.2 (*s*)	H-4’, H-2’
N-H	-	8.9 (*br s*)	-

Based on spectroscopic data and literature survey, compound **52** is expected to be an indole alkaloid with the following structure.

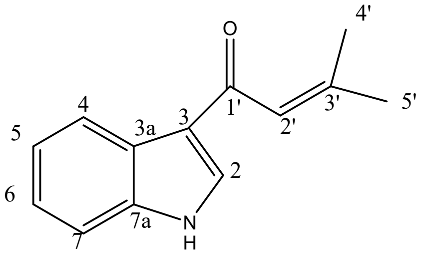


Fig. 3: Proposed structure for BA2 or compound **52**.

The ^{1}H and ^{13}C NMR data of compound **52** showed comparable results with the literature (Vijaya et al., 1993) but the assignments at C-2 and C-2’ interchanged (Table 5). In the literature, C-2 and C-2’ were assigned at δ$_C$ 123.6 and 130.7 respectively while in the case of compound **52**, the 2D NMR results showed that the assignments of C-2 and C-2’ should be reversed. That is C-2 and C-2’ are reassigned at δ$_C$ 122.9 and δ$_C$ 131.0 respectively.

Table 5: Comparison of the ^{13}C NMR and ^{1}H of compound **52** with the literature.

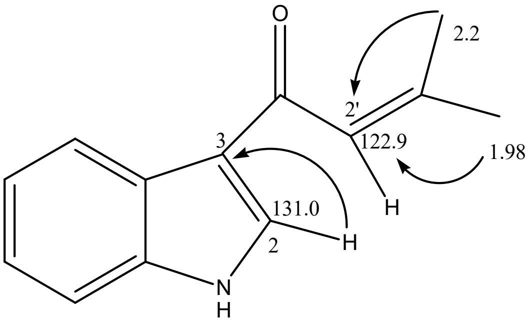
C.No.	^{13}C, δ$_C$	^{13}C, δ$_C$ (Vijaya et al., 1993)	^{1}H, δ$_H$	^{1}H, δ$_H$ (Vijaya et al., 1993)	^{1}H, δ$_H$ (Ernest et al., 1986)
2	131.0 *(*d*)	123.6*(*d*)	7.75 (*d*)	7.84 (*d*)	7.81 (*d*)
3	120.0 (*s*)	119.9 (*s*)	-	-	-
3a	126.3 (*s*)	125.9 (*s*)	-	-	-
4	122.8 (*d*)	122.7 (*d*)	8.47 (*m*)	8.47 (*m*)	7.1-8.6 (*m*)
5	123.0 (*d*)	122.5 (*d*)	7.25-7.3 (*m*)	7.34 (*m*)	‘’
6	123.9 (*d*)	122.5 (*d*)	‘’	‘’	‘’
7	111.7 (*d*)	111.4 (*d*)	‘’	‘’	‘’
7a	136.8 (*s*)	136.5 (*s*)	-	-	-
1’	188.1 (*s*)	187.7 (*s*)	-	-	-
2’	122.9* (*d*)	130.7*(*d*)	6.6 (*s*)	6.64 (*s*)	6.61 (*s*)
3’	153.1 (*s*)	152.7 (*s*)	-	-	-
4’	28.1 (*q*)	27.7 (*q*)	1.98 (*s*)	1.98 (*s*)	1.97 (*s*)
5’	21.2 (*q*)	20.9 (*q*)	2.20 (*s*)	2.26 (*s*)	2.24 (*s*)
N-H	-	-	8.90 (*br s*)	9.09 (*br s*)	-

* Interchanged resonances (see the discussion part)

In the HSQC NMR spectrum, the two vinylic protons at δ_H 6.6 (*s*) and 7.75 (*d*) correlated well with δ_C 122.9 (C-2') and 131.0 (C-2) respectively.

In addition to the above information, based on the HMBC data the following three points were observed and led to the reassignment of C-2 and C-2' of the literature values as shown on Table 5 (Vijaya et al., 1993) (Table 4).

1. The two methyl singlet protons at δ_H 1.98 and 2.20 correlated well with C-2' (δ_C 122.9) but not with C-2 (δ_C 131.0).



2. There was no correlation observed between C-2 (δ_C 131.0) and any other neighboring protons.
3. The vinylic proton, H-2 (δ_C 131.0) showed correlation with a quaternary carbon C-3 (δ_C 120.0).

Therefore, based on spectroscopic data and literature survey, the structure of the compound **52** could be as shown on Fig. 3, (3-(3-methyl-1-oxo-2-butenyl)) 1H indole which has not been reported from any *Bruce*a species.

2.2. Partial Characterization of BA7B or compound 54

The compound BA7B was isolated as yellowish red solid. Analytical thin layer chromatogram was run for BA7B ($R_f = 0.6$) in eluting with solvent system IX (see Table 11). After heating the sprayed plate with 1% vanillin in H_2SO_4, yellow colored spot was observed in the chromatogram (Fig. 4).

Fig. 4: Analytical thin layer chromatogram of BA7B.

The IR (KBr) spectrum of the compound showed a broad OH stretching band at 3376 cm^{-1}, and a conjugated C=O stretching band at 1655 cm^{-1}.

In the UV spectrum (MeOH) of the compound, two strong bands at 354 nm and 213 nm were observed due to bathochromic shifts on the cyclic enones and aromatic rings respectively.

Inspection of the ^{1} H NMR spectrum (Table 6) of the compound showed the presence of aromatic protons between δ_H 6.2 (*s*) and 7.6 (*m*). Two anomeric doublet protons were also observed at δ_H 5.32 and 5.41.

In the ^{13}C and DEPT-135 (Table 6) spectra, the resonances between δ_C 60.9 and 78.3 for ten carbons, and at δ_C 101.7 and 102.8 for the two anomeric carbons with totalizing twelve carbons, confirmed the presence of two glucopyranoside moieties.

Signals for 22 quaternary carbons appeared on the ^{13}C NMR spectrum. These include two α,β- unsaturated carbonyl carbons at δ_C 178.29 and 178.25, oxygenated carbons at δ_C 134.3 and 134.2, 162.01 and 162.03, 165.20 and 165.15, 157.20 and 157.21, 145.64 (two carbons), 149.35 and 149.31, 157.12 and 157.09 and the remaining four carbons at δ_C 99.6, 104.7 and 104.8 (overlapping).

Table 6: ^{1}H, ^{13}C, and HMBC (^{1}H →^{13}C) data of compound **54** in DMSO-*d*$_6$.

C.NO	**δ$_C$**	**δ $_H$**	**HMBC (^{1}H → ^{13}C) correlations**
2, 2’’	157.12 (*s*),157.09 (*s*) ɸ	-	H-2’, H-2’’’
3, 3’’	134.33 (*s*), 134.16 (*s*) ɸ	-	H-1’’’’,H-1’’’’’
4, 4’’	178.29 (*s*), 178.25 (*s*) ɸ	-	H-6, H-8’’
5, 5’’	162.01 (*s*), 162.03 (*s*)	-	H-6
6, 6’’	99.63 (*d*),104.7(104.8) ɸ (*s*) *	6.20 (*s*)	-
7, 7’’	165.20 (*s*), 165.15 (*s*) ɸ	-	H-6, H-8’’
8, 8’’	99.62 (*s*), 94.5 (*d*) *	6.40(*s*)	-
9, 9’’	157.20 (*s*), 157.21 (*s*) ɸ	-	H-8’’
10, 10’’	104.7(*s*), 104.8 (*s*)‡ ɸ	-	H-6, H-8’’
1’, 1’’’	121.91(*s*), 122.00 (*s*) ɸ	-	H-2’, H-2’’’
2’, 2’’’	116 (*d*)‡	6.80 (*t*)	-
3, 3’’’	145.64 (*s*)‡	-	H-2’,H-2’’’, H-5’’’, H-6’’’
4’, 4’’’	149.35 (*s*), 149.31 (*s*) ɸ	-	H-2’, H-2’’’, H-5’’’
5’, 5’’’	117.09 (*d*), 116.87 (*d*) ɸ	7.55 (*m*)	-
6’, 6’’’	122.47 (*d*), 122.72 (*d*) ɸ	7.55 (*m*), 7.60 (*m*)	-
1’’’’, 1’’’’’	101.8 (*d*), 102.8 (*d*)	5.41 (*d*), 5.32 (*d*) *	¥
2’’’’, 2’’’’’	72.1(*d*), 74.0 (*d*)	¥	‘’
3’’’’, 3’’’’’	74.9 (*d*), 76.6 (*d*)	‘’	‘’
4’’’’, 4’’’’’	68.8 (*d*), 70.7 (*d*)	‘’	‘’
5’’’’, 5’’’’’	77.3 (*d*), 78.3 (*d*)	‘’	‘’
6’’’’, 6’’’’’	60.9 (*t*), 61.8 (*t*)	‘’	‘’

ɸ interchangeable resonances
* respectively
‡superimposed resonances
¥ obscured by the solvent peaks

Further inspection of the 2D NMR spectra showed the following correlations. In the HSQC spectrum, the two doublet anomeric protons at δ$_H$ 5.41 and 5.32 showed correlations with the two anomeric carbons at δ$_C$ 101.8 and 102.8 of C-1’’’’ and C-1’’’’’ respectively. The two singlet protons at δ$_H$ 6.20 and 6.40 were also correlated with C-6 and C-8’’ (δ$_C$ 99.6 and 94.5). Moreover the triplet aromatic proton at δ$_H$ 6.8, and the multiplets at δ$_H$ 7.55 and 7.60 showed correlations with C-2’ and 2’’’, 5’, 5’’’ and 6’, and 6’’’ respectively.

The following partial structures were suggested based on the correlations observed in the HMBC spectrum (Table 6).

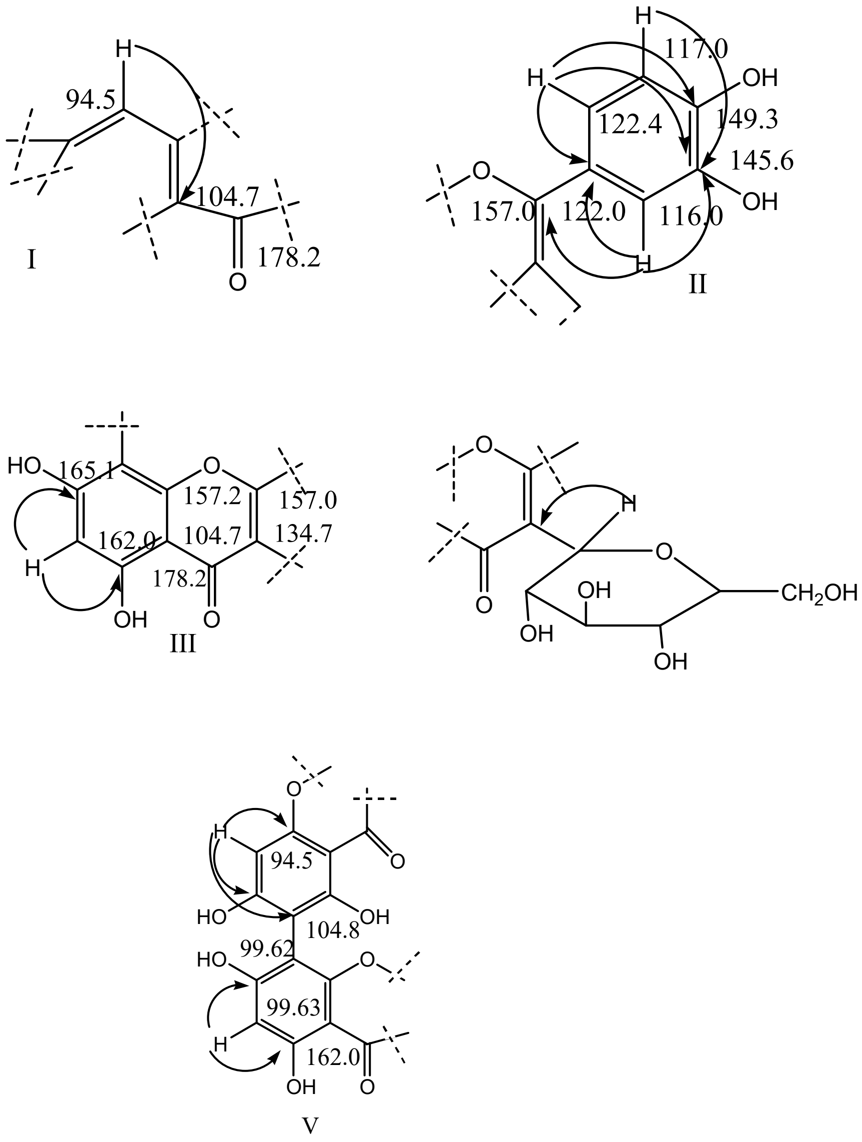


Fig. 5: Selected HMBC correlations and partial structures for compound **54.**

The assignments of the ^{13}C of the glucopyranosyl moiety was based on comparison with quercetin-3-*O*-glucose or BA7 (which was isolated and partially characterized by another co-worker in our research group. The ^{13}C NMR of quercetin-3-*O*-glucose (Harborne, 1982 and Markham et al., 1978) and compound **54** are compared in Table 7.

Based on 1 and 2D NMR spectra and comparing with the literature, compound **54** is expected to be a glycosidic biflavonoid which is probably a dimer of quercetin-3-*O*-glucose (Fig. 6).

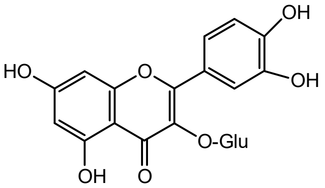


Fig. 6: Structure of quercetin-3-*O*-glucose, compound **53** or BA7.

Table 7: Comparison of the ^{13}C NMR of compound **53** and compound **54.**

C.No.	δ$_C$, compound **54**	δ$_C$, compound **54** (Harborne, 1982 and Markham et al., 1978)
2, 2''	157.12 (*s*),157.09 (*s*) $^{\Phi}$	156.5
3, 3''	134.33 (*s*), 134.16 (*s*) $^{\Phi}$	133.7
4, 4''	178.29 (*s*), 178.25 (*s*) $^{\Phi}$	177.6
5, 5''	162.01 (*s*), 162.03 (*s*)	161.3
6, 6''	99.63 (*d*),104.7(104.8) $^{\Phi}$ (*s*) *	98.8
7, 7''	165.20 (*s*), 165.15 (*s*) $^{\Phi}$	164.2
8, 8''	99.62 (*s*), 94.5 (*d*) *	93.6
9, 9''	157.20 (*s*), 157.21 (*s*) $^{\Phi}$	156.5
10, 10''	104.7(*s*), 104.8 (*s*)ǂ $^{\Phi}$	104.2
1', 1'''	121.91(*s*), 122.00 (*s*) $^{\Phi}$	121.6
2', 2'''	116 (*d*)ǂ	115.3
3, 3'''	145.64 (*s*)ǂ	144.8
4, 4'''	149.35 (*s*), 149.31 (*s*) $^{\Phi}$	148.5
5', 5'''	117.09 (*d*), 116.87 (*d*) $^{\Phi}$	116.5
6', 6'''	122.47 (*d*), 122.72 (*d*) $^{\Phi}$	121.4
1'''', 1'''''	101.8 (*d*), 102.8 (*d*) $^{\Phi}$	101.4
2'''', 2'''''	72.1(*d*), 74.0 (*d*) $^{\Phi}$	74.3
3'''', 3'''''	74.9 (*d*), 76.6 (*d*) $^{\Phi}$	76.8
4'''', 4'''''	68.8 (*d*), 70.7 (*d*) $^{\Phi}$	70.3
5'''', 5'''''	77.3 (*d*), 78.3 (*d*) $^{\Phi}$	77.5
6'''', 6'''''	60.9 (*t*), 61.8 (*t*) $^{\Phi}$	61.3

$^{\Phi}$ interchangeable resonances
* respectively
ǂsuperimposed resonances

From literature survey, the common position of attachments and the corresponding shift changes for ^{13}C resonance was studied. Some of the common positions of attachments observed were C-8, C-6 and C-3’ of the monomer and whenever substitution occurs, down field shift by about +4 ppm were common (Markham et al., 1978).

For compound **54**, the position of dimerization is expected to be at C-8, and C-6’’ based on literature survey, and NMR data. From 1D NMR, the ^{1}H spectrum showed the presence of two singlet protons at δ_H 6.2 and 6.4 (C-6 and C-8’’ respectively). The common couplings always observed between the two protons (H-6 and H-8) on the A-ring of quercetin was not shown in the case of compound **54**. This may imply the substitution of one of the two protons by another group (Ahmed et al., 2000). In addition, from ^{13}C and DEPT-135 spectra, one quaternary carbon observed at δ_C 99.62, which shifts probably by +4 ppm from the lower value which is observed at around δ_C 94.5. This could be due to the replacement of H-8 by another group. Based on this information, C-8 could be the position of substitution (Fig. 7).

The HMBC spectrum of the compound was indicated that the quaternary carbons at 104.8 and 104.7 correlated with H-8’’ and H-6. This is probably due to the correlation between one of the three quaternary carbons with the proton H-8’’, which is shifted from 99.63 to 104.7 (104.8) due to the replacement of the proton by another group. Based on the above spectroscopic data, the two monomer could be attached on C-8 of one and C-6’’ of the other quercetin-3-*O*-glucose.

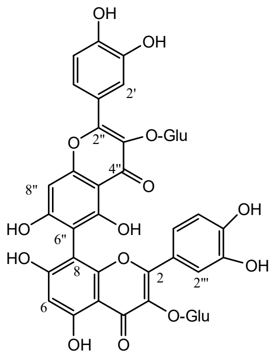


Fig. 7: Proposed structure for BA7B or compound **54**.

Therefore, based on the above data, the structure of compound (BA7B) could be a biflavonoid of quercetin-3-*O*-glucose (Fig. 7). To the best of our knowledge which has not been reported from the genus or any other plant.

2.3. Partial Characterization of BA5 and BA6

Both BA5 and BA6 were obtained as a yellowish white solid with a melting point of 195-198 °C and 188-191 °C respectively (Table 8). Their TLC (R_f =0.6 for BA5 and R_f=0.5 for BA6) run with $CHCl_3$/MeOH at 9:1, showed blue coloration after spraying with 1% vanillin in H_2SO_4 as shown in the following chromatogram. They exhibit the same molecular ion peak $[M+Na]^+$ at m/z 791 in the ESIMS corresponding to $C_{36}H_{48}O_{18}$, and the fragment ion peak observed at m/z 733 probably due to the loss of acetoxy group.

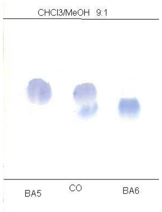


Fig. 8: Analytical thin layer chromatogram of BA5 and BA6

The IR (KBr) spectra of the two compounds indicate (Table 8) the presence of OH, ester functional groups, α,β-unsaturated carbonyl group, unsaturated double bond and C-O stretchings.

In the UV spectra (Table 8), two bands were observed at 217 nm and 261 nm for BA5 and at 229 nm and 263 nm for BA6.

Table 8: Comparison of mp, IR, UV/Vis and MS of BA5 and BA6 with the literature.

Parameters	BA5	BA6	Yadanzioside G (**51)**	
			Toshiro et al., 1985	Noshiro et al., 1987
mp, °C	195-198.5	188-191.4	180-185	180-185
MS $[M+Na]^+$, m/z	791	791	791	791
IR (KBr), cm^{-1}	3401, 1738, 1681, 1640, 1064	3420, 1735, 1680, 1641, 1073	3450, 1740, 1685, 1645, 1075	3420,1738, 1682,1650, 1070
UV, nm	217, 261	229, 263	222, 253	*

*not measured

Inspection of the ^{1} H NMR spectrum of BA5 (Table 9) showed the presence of two vinylic, methyl and methine protons. In addition to that one doublet anomeric proton also observed at δ_H 4.62. In the ^{13}C and DEPT-135 (Table 9) of the compound BA5, the presence of glucopyranoside moiety was confirmed from δ_C 101.2 attributed to the anomeric carbon, one methylene carbon at δ_C 61.3 due to C-6 of the sugar moiety and other four methine carbons at δ_C 70.1, 73.9, 77.8, and 77.9. The five carbonyl carbons observed between δ_C 194.9 and 167.6 (Table 9) were assigned to C-3, C-16, C-20, C-1' and C-8'. One oxymethine carbon also observed at δ_C 83.3 due to C-7 which is common for most C-20 quassinoids (Toshiro et al., 1985). Furthermore the two quaternary carbons observed at δ_C 82.2 and 82.7 were assigned to C-13 and C-4' respectively.

Inspection of the 2D NMR spectra of BA5 showed the following correlations. In the HSQC spectrum, the vinylic protons appearing at δ_H 6.6 and 5.6 correlate with δ_C 130.0 and 113.3 which were assigned to C-1 and C-2' respectively. The anomeric doublet proton observed at δ_H 4.62 also showed correlation with C-1' appearing at δ_C 101.2. (see Table 9 for detailed correlations).

Based on the observed HMBC correlations (Table 9), the following partial structures were suggested. The rings A and B (partial structure VII) of the compound could be assembled from the suggested fragments (I-VI).

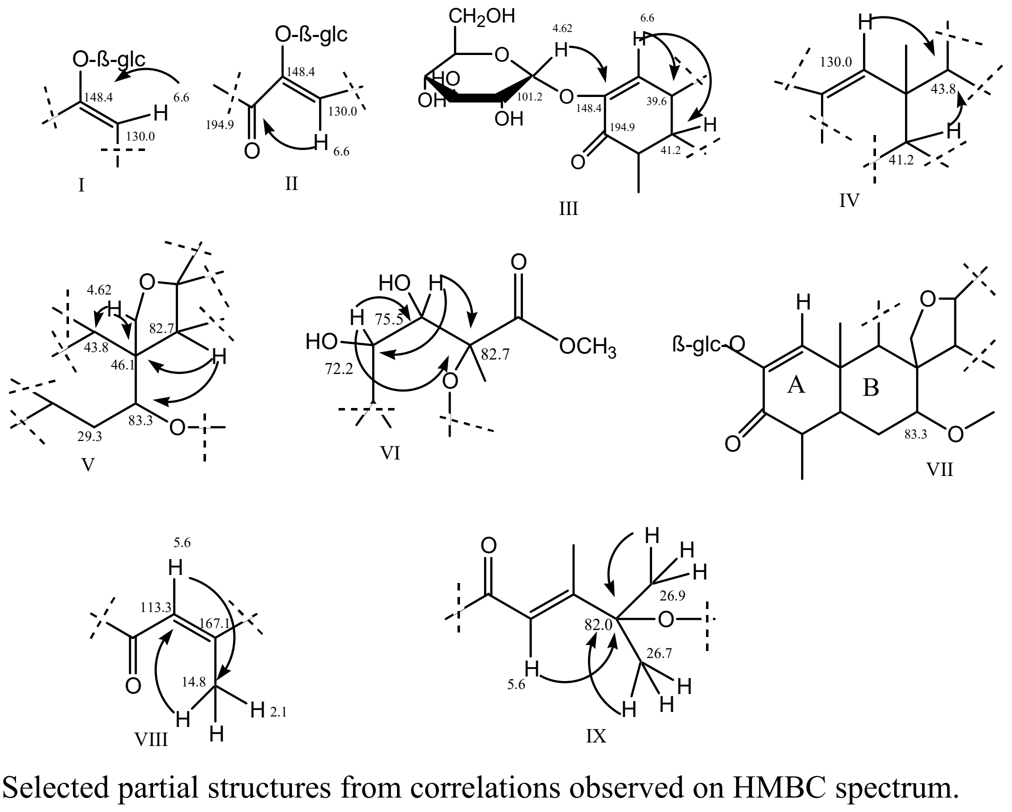


Fig. 9: Selected partial structures from correlations observed on HMBC spectrum.

Table 9: ^{1}H, ^{13}C, and HMBC (^{1}H →^{13}C) NMR data of BA5 in DMSO-d_6.

C.No.	**BA5, δ_C**	**BA5, δ_H**	**BA5, HMBC ^{1}H → ^{13}C**
1	130.0 (*d*)	6.6 (*s*)	-
2	148.4 (*s*)	-	H-1, H-1’’
3	194.9 (*s*)	-	H-1
4	43.9 (*d*)	-	H-1
5	41.2 (*d*)	2.5 (*m*)	H-1
6	29.9 (t)	1.85 (*m*)	-
7	83.3 (*d*)	4.9 (*m*)	H-14
8	46.1 (*s*)	-	H-17, H-14
9	43.8 (*d*)	1.89 (*m*)	H-1, H-17, H-5
10	39.6 (s)	-	H-1
11	72.4 (*d*)	4.3 (*m*)	H-12
12	75.5 (*d*)	4.2 (*m*)	H-11
13	82.7 (*s*)	-	H-11, H-12
14	52.9 (*d*)	3.7 *	-
15	70.2 (*d*)	3.22*	-
16	170.0 (s)	-	-
17	73.9 (*t*)	4.62 (*m*), 3.7 (*m*)	-
18	13.3 (*q*)	1.01 (*s*)	H-5
19	17.1 (*q*)	1.9 (*d*)	-
20	170.7 (*s*)	-	-
OMe	52.8 (*d*)	3.7*	-
1’	167.6 (s)	-	-
2’	113.3 (s)	5.6 (*s*)	H-5’
3’	168.1 (*s*)	-	H-2’, H-5’, H-6’, H-7’
4’	82.0 (*s*)	-	H-2’
5’	14.8 (*t*)	2.1 (*s*)	-
6’	26.9 (*t*)	1.42 (*s*)	-
7’	26.7 (*t*)	1.42 (*s*)	-
8’	170.6 (s)	-	-
9’	21.5 (*t*)	1.01 (*s*)	-
1’’	101.2 (*d*)	4.62 (*d*)	-
2’’	73.9 (*d*)	3.2*	-
3’’	77.9 (*d*)	3.2*	-
4’’	70.1(*d*)	3.22*	-
5’’	77.8 (*d*)	3.2*	-
6’’	61.3 (*t*)	3.75*	-

*overlapped with other peaks (multiplicity not determined)

Based on 1 and 2-D NMR spectra and other spectroscopic data and comparison with literature (Table 10), the structure of BA5 is similar to the previously isolated compound from *Brucea javanica* (Toshiro et al., 1985) and *Brucea antidysenterica* (Narihiko et al., 1987) which is known as yadanzioside G (**51)** (methyl 2-(*β*-D-glucopyranosyloxy)-13*β*,20-epoxy-15*β*-[(2E)-4-acetoxy-3,4-dimethyl-2-pentenoyloxy]-11*β*,12*α*-dihydroxy-3,16-dioxo-1-picrasen-21-oate).

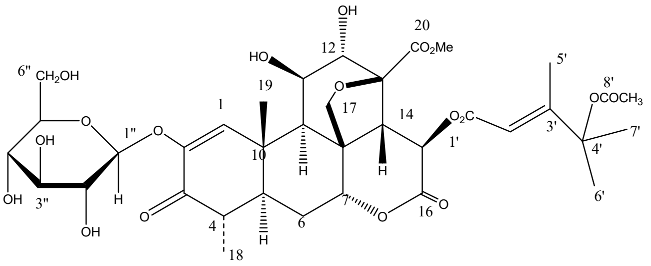


Fig. 10: Structure of yadanzioside G (**51**)

Table 10: Comparison of the ^{13}C NMR and ^{1}H of BA5 and the literature values**.**

C.No.	BA5, δ_C	Compound **51,** (Narihiko et al., 1987), δ_C	Compound **51,** (Toshiro et al., 1985), δ_C
1	130.0 (*d*)	129.3 (*d*)	129.7 (*d*)
2	148.4 (*s*)	148.9 (*s*)	148.8 (*s*)
3	194.9 (*s*)	194.5 (*s*)	194.7 (*s*)
4	43.9 (*d*)	43.8 (*d*)	43.9 *d*)
5	41.2 (*d*)	40.3 (*d*)	40.4 (*d*)
6	29.9 (*t*)	30.1 (*t*)	30.1 (*t*)
7	83.3 (*d*)	83.5 (*d*)	83.5 (*d*)
8	46.1 (*s*)	46.6 (*s*)	46.6 (*s*)
9	43.8 (*d*)	41.4 (*d*)	41.4 (*d*)
10	39.6 (s)	39.6 (s)	39.6 (s)
11	72.4 (*d*)	73.6 (*d*)	73.4 (*d*)
12	75.5 (*d*)	76.0 (*d*)	76.0 (*d*)
13	82.7 (*s*)	82.6 (*s*)	82.6 (*s*)
14	52.9 (*d*)	52.6 (*d*)	50.2 (*d*)
15	70.2 (*d*)	71.3 (*d*)	68.7 (*d*)
16	170.0 (*s*)	168.1 (*s*)	168.0 (*s*)
17	73.2 (*t*)	73.7 (*t*)	73.7 (*t*)
18	13.3 (*q*)	12.6 (*q*)	12.5 (*q*)
19	17.1 (*q*)	18.0 (*q*)	18.0 (*q*)
20	170.7 (*s*)	171.1 (*s*)	171.7 (*s*)
OMe	52.8 (*d*)	50.2 (*d*)	52.6 (*d*)
1’	167.6 (*s*)	165.7 (s)	165.7 (*s*)
2’	113.3 (*s*)	113.6 (*s*)	113.7 (*s*)
3’	168.1 (*s*)	167.1 (s)	169.5 (*s*)
4’	82.0 (*s*)	82.3 (*s*)	82.3 (*s*)
5’	14.8 (*q*)	14.5 (*q*)	14.5 (*q*)
6’	26.9 (*q*)	26.4 (*q*)	26.4 (*q*)
7’	26.7 (*q*)	26.8 (*q*)	26.8 (*q*)
8’	170.6 (*s*)	169.5 (*s*)	163.5 (*s*)
9’	21.5 (*q*)	21.4 (*q*)	21.4 (*q*)
1’’	101.2 (*d*)	102.1 (*d*)	102.0 (*d*)
2’’	73.9 (*d*)	74.7 (*d*)	74.6 (*d*)
3’’	77.9 (*d*)	79.0 (*d*)	78.8 (*d*)
4’’	70.1 (*d*)	71.3 (*d*)	71.3 (*d*)
5’’	77.8 (*d*)	78.5 (*d*)	78.5 (*d*)
6’’	61.3 (*t*)	62.3 (*t*)	62.3 (*t*)

Comparing with the literature and using the above suggested fragments, the structure of BA5 proposed as shown on next page.

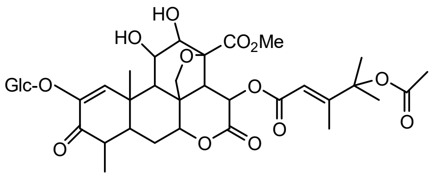


Fig. 11: Proposed structure for BA5 or compound **55**.

The other compound, BA6 showed closer R_f value, similar coloration when sprayed with 1% vanillin in H_2SO_4 , comparable 1D NMR (Table 11), IR and MS (Table 8) spectra.

Table 11: Comparison of the ^{13}C NMR of BA5 and BA6**.**

C.No.	BA5, δ_C	BA6, δ_C
1	130.0 (*d*)	128.4 (*d*)
2	148.4 (*s*)	145.9 (*s*)
3	194.9 (*s*)	200.7 (*s*)
4	43.9 (*d*)	44.1 *d*)
5	41.2 (*d*)	36.4 (*d*)
6	29.9 (*t*)	28.3 (*t*)
7	83.3 (*d*)	83.3 (*d*)
8	46.1 (*s*)	46.2 (*s*)
9	43.8 (*d*)	38.4 (*d*)
10	39.6 (s)	36.4 (s)
11	72.4 (*d*)	74.0 (*d*)
12	75.5 (*d*)	76.8 (*d*)
13	82.7 (*s*)	82.6 (*s*)
14	52.9 (*d*)	52.3 (*d*)
15	70.2 (*d*)	73.6 (*d*)
16	170.0 (s)	168.9 (s)
17	73.2 (*t*)	73.4 (*t*)
18	13.3 (*q*)	13.8 (*q*)
19	17.1 (*q*)	18.4 (*q*)
20	170.7 (*s*)	170.9 (*s*)
OMe	52.8 (*d*)	50.2 (*d*)
1’	167.6 (*s*)	168.4 (*s*)
2’	113.3 (*s*)	112.9 (*s*)
3’	168.1 (*s*)	168.8 (*s*)
4’	82.0 (*s*)	82.2 (*s*)
5’	14.8 (*q*)	15.9 (*q*)
6’	26.9 (*q*)	25.8 (*q*)
7’	26.7 (*q*)	25.5 (*q*)
8’	170.6 (s)	170.5 (*s*)
9’	21.5 (*q*)	20.6 (*q*)
1’’	101.2 (*d*)	100.8 (*d*)
2’’	73.9 (*d*)	75.7 (*d*)
3’’	77.9 (*d*)	78.5 (*d*)
4’’	70.1 (*d*)	70.5 (*d*)
5’’	77.8 (*d*)	77.2 (*d*)
6’’	61.3 (*t*)	61.6 (*t*)

Based on the above information the two compounds are expected to be diastereoisomers, that is they could probably vary at one of the stereogenic centers of the compounds. In the ^{13}C NMR, the observed resonance differences in ring A and C-9 (δ_C 43.8 for BA5 and δ_C 38.4 for BA6) probably indicate the glucopyranosyl moiety attached on C-2 of the compound could have opposite stereochemistry from BA5**.**

3. Experimental

3.1. General

Melting points were determined in capillary tubes with a digital electrothermal melting point apparatus. IR spectra were recorded with a Perkin-Elmer BX Spectrometer (400-4000 cm^{-1}) in KBr. UV spectra were determined using a GENESY'S 2PC Spectrometer (200-400 nm) at room temperature. All NMR experiments were recorded using a Bruker 400 MHz Avance NMR Spectrometer. ^{1}H and ^{13}C NMR were measured at 400 MHz and 100 MHz respectively in $CDCl_3$, MeOH-d_4, and DMSO-d_6 with TMS as an internal standard.

3.2. Plant Material

The seeds of *B. antidysenterica* were collected by Ermias Dagne, African Laboratory of Natural Product, Department of Chemistry, Addis Ababa University around Mogli Mountain 40 km west of Addis Ababa, in March 2004.

3.3. Solvent Systems

Different solvent systems were used for TLC, PTLC , MPLC, FCC and LH-20 Sephadex as shown in Table 12.

Table 12: Solvent systems used for TLC, PTLC, MPLC, FCC and LH-20 Sephadex.

Technique used	Solvent	Proportion
TLC	Solvent I	$CHCl_3$: MeOH (9:1)
	Solvent II	$CHCl_3$: MeOH (8:2)
Prep. TLC	Solvent III	$CHCl_3$: MeOH (8.5: 1.5)
FCC	Solvent IV	EtOAc: MeOH (8:2)
	Solvent V	$CHCl_3$: MeOH (9:1)
	Solvent VI	$CHCl_3$: MeOH (8.5: 1.5)
MPLC	Solvent VII	EtOAc: MeOH (9:1)
	Solvent VIII	EtOAc: MeOH (8:2)
Sephadex LH -20	Solvent IX	CH_2Cl_2: MeOH (1:1)

3.4. Coding system

B stands for the genus name *Brucea*, and A stands for species name *antidysenterica*. The numbers indicate the R_f of the compound in the TLC starting from the largest values. Thus BA2 stands for the second compound, BA5 for the fifth, BA6 for the sixth and BA7B the seventh compound.

3.5. Extraction and Isolation

3.5.1. Isolation of BA5 and BA6

The pulverized seeds of *B. antidysenterica* (1 kg) was extracted with 90% aqueous methanol three times and then defatted with petrol (300 ml) three times. The 90% aqueous methanol extract was diluted by partitioning combined extract to 1:1 by using water. The 50% aqueous methanol was extracted with EtOAc three times. After drying, about 2 g of 100% EtOAc crude extract was obtained. The 2 g EtOAc extract was subjected to FCC on silica gel to collect three fractions by using Petrol/EtOAc (1:1), 100% EtOAc and EtOAc/MeOH (1:1) and 60 mg, 40 mg and 70 mg of fractions were obtained respectively. The last fraction which was mainly BA5 and BA6 was subjected to Flash Column Chromatography and eight fractions were collected using CH_2Cl_2 and MeOH by increasing polarity. The fifth and sixth fractions (collected with CH_2Cl_2/MeOH 9:1) were combined and subjected to PTLC using CH_2Cl_2/MeOH (85:15) to give light yellowish compounds BA5 (4 mg) and BA6 (1.4 mg).

Compound BA5 (77-43L): BA5 was obtained as a light yellowish solid, mp 195-198 °C; IR (KBr) ν_{max} cm^{-1}: 3401, 1738, 1681, 1640, 1064; ESIMS [M+Na]$^+$: 791 for $C_{36}H_{48}O_{18}$; UV (MeOH) λ_{max}: 217nm and 261nm; ^{1}H and ^{13}C NMR (400 MHz in DMSO-*d*$_6$) (See Table 9).

Compound BA6 (77-44A): BA6 was obtained as a light yellowish solid, mp 188-191°C); IR (KBr) ν_{max} cm^{-1}: 3420, 1735, 1680, 1641, 1073; [M+Na]$^+$: HR-ESIMS 791.2733 for $C_{36}H_{48}O_{18}$, calculated 768.757; UV (MeOH) λ_{max}: 229 nm and 263 nm; ^{1}H and ^{13}C NMR (400 MHz in MeOH-*d*$_4$) (See Table 9).

3.5.2. Derivatization of compound BA5

Derivatization reaction was performed by taking about 1.5 mg of compound BA5 and adding a mixture of 5 drops of 6M of HCl and 1 ml of MeOH. The mixture was allowed to react at room temperature while stirring for about 20 minutes. The TLC of the resulting product (BA5D) was run with $CHCl_3$/MeOH (7:3). The compound showed blue coloration after spraying with 1% vanillin. The major spot observed on BA5D is expected to be the hydrolyzed product of compound BA5

3.5.3. Isolation of BA7B and BA2

The pulverized seeds of *B. antidysenterica* (1.5 kg) was soaked three times with 90% aqueous ethanol at room temperature, filtered and then concentrated under reduced pressure (40°C) to give a mixture of reddish tar and oil. After decanting the oil about 30 g (2%) of the crude extract (reddish tar) was obtained. 12 g of the crude extract was partitioned sequentially with petrol, CH_2Cl_2 after the addition of water to dilute to 70% and with EtOAc after 50 % dilution resulting 900 mg (0.06 %) of the CH_2Cl_2 extract (89-53A) and 500 mg (0.03 %) of the EtOAc extract (89-53C).

The CH_2Cl_2 extract was subjected to FCC and after purification, compound BA2 was isolated.

The EtOAc extract (89-53C) of the plant was subjected to Separo AB using mixtures of CH_2Cl_2, EtOAc and MeOH successively with increasing polarity to collect six fractions. The TLC of the fourth and the fifth fractions showed the presence of BA5, BA6 and BA7B after spraying with 1% vanillin in H_2SO_4.

The fourth and fifth fractions collected from 89-53C were combined (250 mg) and subjected to FCC using 230-400 mesh size silica gel (12 g) for column packing. CH_2Cl_2, EtOAc and MeOH were used with increasing polarity and four fractions were collected. The TLC (solvent system IX) of the third fraction indicated the presence of yellow spot (BA7B) after spraying with 1% vanillin in H_2SO_4.

Compound BA7B (89-50A): Yellowish brown solid; IR (KBr) ν_{max} cm^{-1}: 3376, 1655, 1506, 1444; UV (MeOH) λ_{max}: 354 and 213 nm; ^1H and ^{13}C NMR data is shown on Table 6.

The CH_2Cl_2 extract of the crude obtained from 90% aqueous ethanol was subjected on FCC using CH_2Cl_2 and MeOH gradients of increasing polarity. The first fraction collected with 100% CH_2Cl_2 contains BA2 and other compounds. Through purification on PTLC a yellow solid BA2 has been isolated (1 mg).

Compound BA2: BA2 was obtained as a light yellowish solid and recrystallized in methanol to give needle crystals; IR (KBr) ν_{max} cm^{-1}: 3275, 643, 1589, 1473; HR-ESIMS m/z 222.089 $[M+Na]^+$, calculated 199.252 for $C_{13}H_{13}NO$; ^1H and ^{13}C NMR data is shown in Table 5.

4. Conclusion and Recommendations

This work has demonstrated that the seeds of *Brucea antidysenterica* are rich sources of compounds with good potential for further biological and chemical research. Due to limitation of time certain measurements such optical rotation and other data were not generated for some of the samples. Furthermore, some of the compounds should be converted to simpler derivatives so as to confirm the proposed structures. It is hope that this and other aspects will be continued in the future.

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