PHYTOCHEMICAL STUDIES
ON
MERENDERA SCHIMPERI (*MERENDERA ABYSSINICA*)

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES,
ADDIS ABABA UNIVERSITY, IN PARTIAL FULFILMENT OF THE
REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE
IN CHEMISTRY

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ADDIS ABABA
AUGUST, 1996

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ACKNOWLEDGMENTS

I would like to thank my research advisor Dr. Wendimagegn Mammo for his unreserved advise, follow-up and supervision at all the times in this work.

I am also indebted to Dr. Sebsebe Demissew for his help and guidance in the course of this work.

I wish to thank Dr. Ermias Dagne for his valuable suggestions, for providing me with reference samples and for allowing me to use facilities in his laboratory. I am also indebted to all members of Dr Ermias's research group for their encouragement and support.

I am grateful to my colleagues, Negussie Wodajo, Bitew Fisseha, Adane Bitew, Omer Yaynie, Abebaw Gedefaw and Gonie Tegbaru for their moral support.

Financial support from the Swedish Agency for Research Cooperation with Developing Countries (SAREC) through the School of Graduate Studies is gratefully acknowledged.

The Department of Chemistry, AAU, is acknowledged for providing me with the laboratory facilities and materials.

Ethiopian Health and Nutrition Research Institute (EHNRI) is also acknowledged for its material and financial support.

ABSTRACT

In the course of this work phytochemical investigation of *Merendera schimperi* (Colchicaceae) was conducted.

The genus *Merendera* is distributed in South Europe, in Asia minor, West Asia, North West Africa and North and North-East Africa. This genus is represented by 15 species.

M. schimperi (syn. *M. abyssinica, M. longifolia, M. longispatha*) is the only *Merendera* species found in Ethiopia.

The corm and corm cover of this plant afforded many compounds, out of which structures were proposed for seven compounds based on their 90 MHz ^{1}H-NMR, IR, and LRMS spectra. These include, β-sitosterol (**31**), erythrinasinate (**32**), stigmast-1,4-diene-3-one (**33**), stigmast-4-ene-3-one (**34**), colchicine (**1**), sucrose (**35**), ethylmethylamine oxide (**36**).

Colchicine (**1**) was the most abundant tropolone alkaloid (*ca.* 1.7% of the dry weight of the corm cover) obtained from both the corm and corm cover of the plant. The chloroform extract, in both cases, was rich in colchicine (**1**) as compared to the methanol extract.

2-Demethylcolchicine (**2**) was synthesized from colchicine(**1**) by selectively demethylating the methoxyl group at position 2. This compound was then used

as a reference sample to monitor its presence in the various extracts of *M. schimperi*. It was not detected in any of the extracts.

The non-polar fractions of the extracts of *M. schimperi* afforded erythrinasinate (**32**). This compound has been isolated from a *Merendera* species for the first time. It was, however, obtained from different *Erythrina* species.

The sterols, β-sitosterol (**31**), stigmast-1,4-diene-3-one (**33**) and stigmast-4-ene-3-one (**34**) were found in considerable quantities in both the chloroform and methanol extracts. The presence of these compounds in the extracts of *Merendera* species has not been reported before.

Ethylmethylamine oxide (**36**) was obtained in small quantities from the methanol extract of the corm. The presence of this compound in *Merendera* is reported here for the first time.

In view of the important place of colchicine in modern medicine, it may be possible to propagate a controlled use of *M. schimperi* among the rural community as a substitute for modern and expensive drugs.

1. INTRODUCTION

The study of natural products is a multidisciplinary activity embracing chemistry and a number of areas in biological sciences. It involves isolation, characterization and synthesis of compounds of natural origin.

The use of natural products as medicines, poisons, hallucinogens, stimulants, perfumes, flavouring agents, insecticides, fungicides, plant growth regulating hormones, etc., is well known. The chemical compounds responsible for these activities are often the secondary metabolites of plants or natural products, as they are usually referred to [1].

Natural products are still untapped reservoir of traditional medicines. Especially in the developing countries, they are well known substitutes to modern drugs which may be unavailable and unaffordable. It is also important to note that about 40% of modern drugs are of natural origin [2].

With the advent of modern spectroscopic techniques such as nuclear magnetic resonance (NMR), mass spectrometry, X-ray crystallography, etc., structural elucidation of natural products has become much more facile. Using the modern techniques, the structures of minute quantities of compounds can be established.

Natural product studies, have benefited enormously from the existence of isotopes of elements such as ^{13}C, ^{18}O, ^{15}N, etc. Isotopic marking experiments have contributed a great deal in the establishment of biosynthetic pathways.

Contemporary interest in natural products is turning increasingly to more biological topics such as chemotaxonomy, enzyme studies and chemical ecology. Chemotaxonomy is concerned with the description and classification of plants. If structurally similar compounds derived from different plant species, are shown to share the same biosynthetic pathway, tentative assignment of both species to the same plant genus or family can be made [3]. Therefore, the study of structural inter-relationships and biogenetic origin of natural products is the basis of chemotaxonomy.

Natural product chemists are also engaged in the development of synthetic methods, particularly for those compounds that they find to be medicinally important. In their attempts to develop reliable methods of synthesis, chemists tend to imitate the plants.

2. THE CHEMISTRY OF THE GENUS *MERENDERA*

The recently revised family, Liliaceae in the order *Liliflorae*, was one of the widely distributed families of flowering plants. It consisted of over 250 genera comprising 3700 species, mostly perennial herbs with rhizomes or bulbs [4] . Recently, a major revision of superorders, orders, and families within the monocotyledons was made and this family was sub-divided into several other families. Accordingly, most of the genera earlier classified in the family Liliaceae are now classified under smaller and more homogeneous families [5, 6].

The family Colchicaceae (which was previously included in the family Liliaceae) consists of some 17 genera and 170 species, distributed in South Africa, the Mediterranean area to Asia, and in Australia. The genera *Iphigenia, Gloriosa, Androcymbium, Littonia* and *Merendera* are now categorized under the family Colchicaceae [5, 6].

The genera *Androcymbium, Bulbocodium, Camptorrhiza, Dipidax, Gloriosa, Iphigenia, Littonia, Merendera, Ornithoglossum* and *Sandersonia* (belonging to the old Liliaceae) possess similar chemical constituents [4]. However, during the reclassification of the Liliaceae, these genera have been placed into separate families. It is thus important to look into the chemistries of these genera that would be of some chemotaxonomic significance.

The genus *Merendera* is represented by 15 species in Africa, Europe and Western Asia [*7*].

M. schimperi (syn. *M.abyssinica, M.longifolia, M.longispatha*) is the only *Merendera* species growing in Ethiopia. Its locations are Tigray, Gondar, Gojjam, Wello, Shoa, Arissi, Sidamo, Bale and Harrar [7].

To date, phytochemical studies have been conducted on about eleven *Merendera* species [8]. Tropolone alkaloids constitute the major secondary metabolites [9] in the genus. Colchicine (**1**) is probably the most important of the tropolone alkaloids. Its presence in many species of *Colchicum, Merendera*, and other

genera of the families Liliaceae and Colchicaceae has been documented [10, 11, 12] .

The genus *Merendera* was also found to elaborate homoaporphine alkaloids. Floramultine (**20**) and trigamine-N-oxide (**27**) are typical examples of this class of compounds. Other kinds of compounds obtained from the genus include, amino acids, benzoic acid and its derivatives, polysaccharides and flavonoids [8,13].

There are some reports that showed the colchicine contents of *Colchicum* and *Merendera* species are identical. For example, *M. caucasica* and *M. sobolifera* contain almost identical content of colchicine like *C. autumnale* [14].

M. filifolia is believed to resemble *C. libanoticum, C. cupani* and *C. cornigerum* due to the presence of cornigerine (**29**), colchicine (**1**) and N-formyl-N-deacetylcolchicine (**8**) in all four plants [15].

In *M. robusta*, tropolone alkaloids were found in the leaves. But its colchicine content was very little [16]. In the flowering period, it contains only tropolonic alkaloids and their lumi derivatives [17]. During the fruitage period, the leaves, stalks, and seeds contained 0.4-0.5, 1.33-1.78 and 0.31-0.36% alkaloids, respectively. While from the corms, colchicine (**1**), colchiceine (**12**), β-lumicolchicine (**18**), γ-lumicolchicine (**19**), 2-and 3-demethylcolchicine (**2, 3**), demecolcine (**7**) were isolated [18, 19].

Extraction of *M. sobolifera* seeds with benzene yielded 0.70% of raw colchicine (**1**) which was lowered to 0.45% upon purification [19].

The alkaloid content in the leaves and stalks of *M. jolantae*, after flowering period, was found to be 0.39%. The main alkaloids were, colchicine (**1**), colchamine (demecolcine, **7**) and colchiceine (**12**) [20]. Its corm, harvested at the stage of seed ripening, contained 0.046, 0.14, and 0.010% of neutral, basic and phenolic-basic fractions of alkaloids, respectively. The neutral fraction contained colchicine (**1**) [21]. Table 1 compares the colchicine (**1**) content of some *Merendera* species.

Table 1. Colchicine (**1**) content of some *Merendera* species.

Species	Plant part	Percentage	Reference
M. attica	corms	0.10	[22]
M. caucasica	corms	0.13	[22]
M. sobolifera	corms	0.13	[22]
M. trigina	corms	0.13	[22]
M. bulbocodium	corms	0.02	[23]
M. persica	corms	0.02	[23]
M. robusta	whole plant	0.17	[23]

The highest alkaloid content of *M. raddeana* was found to be at the end of the flowering season [24]. Floramultine (merendrine, bechaunine, **20**), colchicine (**1**) and a number of known bases of related structures were identified [25, 26].

In the corms of *M. kurdica, M. manissadjianii (*syn: *M. trigyna, M. caucasica*) and *M. sobolifera,* the major alkaloid was colchicine (**1**) [27]. In *M. trigyna* (*M. caucasica)* the presence of non-phenolic alkaloids and phenolic alkaloids were reported. But the highest alkaloid content was reported in the leaves [28]. In the above-ground parts of *M. kurdica* 2- and 3-*O*-demethylated derivatives of colchicine predominated over colchicine (**1**) [9].

The corms of *M. persica*, which was collected from India (Kashmir), was found to contain colchicine (**1**) and demecolcine (**7**). The same was true for *M. bulbocodium* collected in Pyrenees [29].

The corms and flowers of *M. bulbocodium* yielded colchicine (**1**), N-formyl-N-deacetylcolchicine (**8**) and tropolone alkaloids with free phenolic groups. But the basic extract of this plant contained only alkaloids lacking tropolone ring [20].

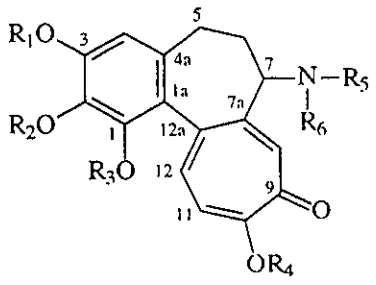
The occurrence of free amino acids were reported in the flowers, leaves, stems and fruits of some *Merendera* species [30]. For example, the presence of 12 amino acids *viz.* tyrosine, alanine, leucine, aspartic acid, lysine, serine, isoleucine, glutamic acid, arginine, phenylalanine, DL-valine and DL-proline were reported from *M. caucasica* [8].

Water soluble polysaccharides such as arabinose, galactose, glucose, mannose, rhamnose and xylose were reported from *Merendera robusta* [13].

The presence of some derivatives of benzoic acid such as, 2-hydroxy-6-methoxy- and 4-hydroxy-3-methoxybenzoic acids and the flavonoid, 2-(3,4-dihydroxy-phenyl)-5,7-dihydroxychromene-4-one (**28**), in *M. kurdica, M. sobolifera* and *M. caucasia* were reported [8].

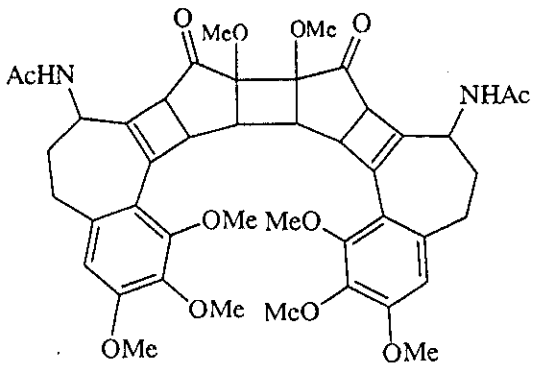
A recent report shows the absence of 2-and 3-demethylcolchicine (**2, 3**) from the fresh material of some *Merendera* species, while they are present in considerable quantity in a material that was slowly dried before extraction. This was attributed to enzymatic liberation [8]. Table 2 shows different compounds that have been obtained from different *Merendera* species.

Table 2. Compounds Isolated form different species of *Merendera*.



No.	Compound	R_1	R_2	R_3	R_4	R_5	R_6	Source	Reference
1.	Colchicine	Me	Me	Me	Me	Ac	H	Several *Merendera* spp	[8, 9, 31, 32]
2.	2-Demethylcolchicine	Me	H	Me	Me	Ac	H	*M. robusta*, *M. caucasica*	[8, 9, 32, 33]
3.	3-Demethylcolchicine	H	Me	Me	Me	Ac	H	*M. caucasica* & several other spp	[8, 31, 34, 35]
4.	N-Deacetylcolchicine	Me	Me	Me	Me	H	H	*M. caucasica* & soveral other spp	[8, 33]
5.	2,3-Didemethylcolchicine	H	H	Me	Me	Ac	H	*M. caucasica*	[8]
6.	3-Demethyl-N-deacetyl-N-formylcolchicine	H	Me	Me	Me	CHO	H	*M. folifolia*	[8]

7.	Demecolcine	Me	Me	Me	Me	Me	H	*M. jolantae, M. persica* & several spp	[8, 9, 32, 36]
8.	N-Formyldemecolcine	Me	Me	Me	Me	Me	HCO	*M. folifolia, M.persica*	[8]
9.	N-Methyldemecolcine	Me	Me	Me	Me	Me	Me	*M. robusta*	[8]
10.	2-Demethyldemecolcine	Me	H	Me	Me	Me	H	*M. persica, M. robusta*	[8]
11.	3-Demethyldemecolcine	H	Me	Me	Me	Me	H	*M. persica, M. robusta*	[8]
12.	Colchiceine	Me	Me	Me	H	Ac	H	*M. robusta, M. jolantae*	[8, 20, 31, 36]
13.	2-Demethylcolchiceine	Me	H	Me	H	Ac	H	*M. robusta*	[8]
14.	3-Demethylcolchiceine	H	Me	Me	H	Ac	H	*M. jolantae*	[8]
15.	N-Deacetylcolchiceine	Me	Me	Me	H	H	H	*M. robusta, M. jolantae*	[8]
16.	Demecolceine	Me	Me	Me	H	Me	H	*M. robusta, M. jolantae*	[8, 9, 33, 36]



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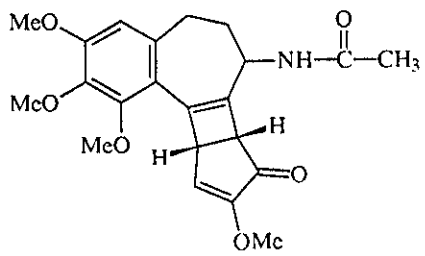

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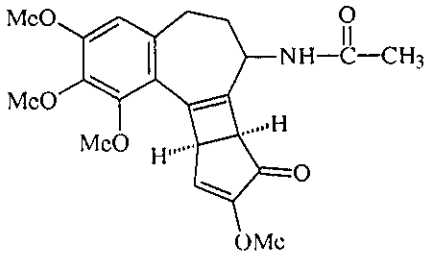

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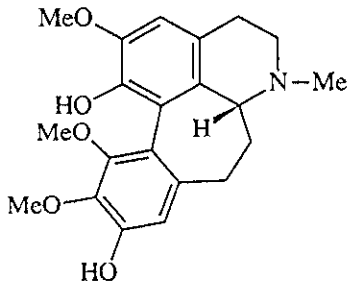

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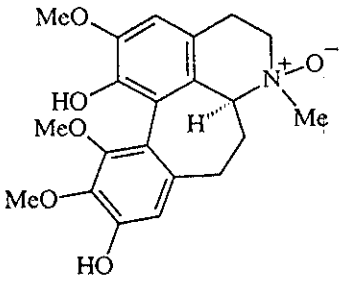

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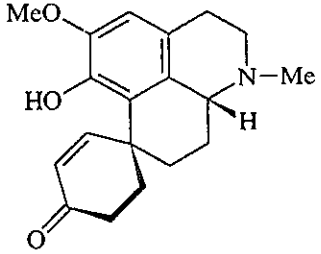

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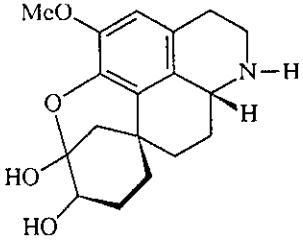

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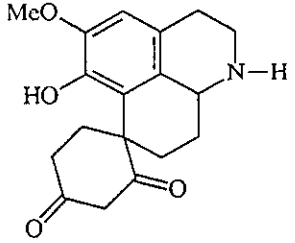

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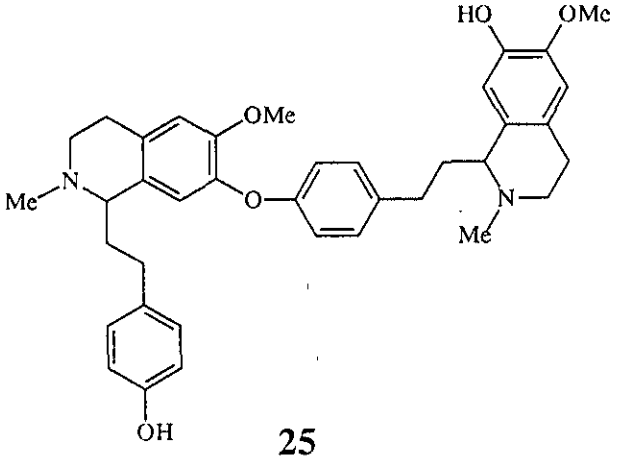

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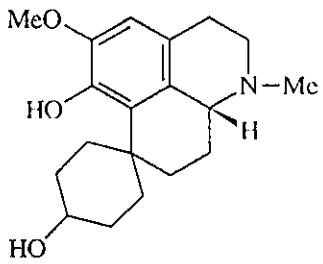

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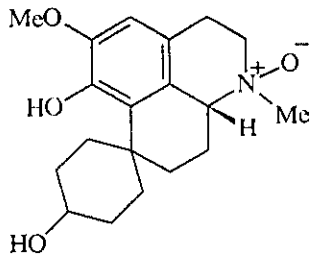

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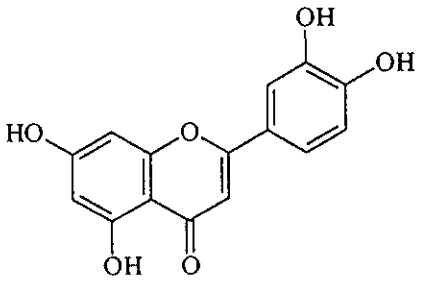

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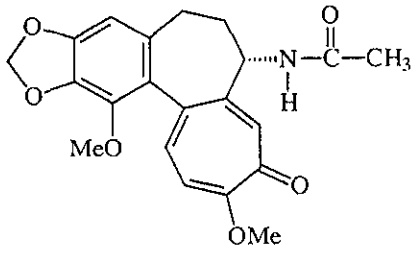

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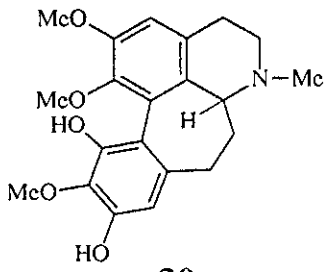

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Table 2. Continued...

No.	COMPOUND	SOURCE	REFERENCES
17.	α-Lumicolchicine*	---	[8]
18.	β-Lumicolchicine	*M. robusta*	[8, 35, 36]
19.	γ-Lumicolchicine	Several *Merendera spp*	[8]
20.	Floramultine	*M. raddeana, M.trigina*	[8, 37]
21.	Merendrine-N-oxide	*M. raddeana*	[8]
22.	Jolantamine	*M. jolantae*	[8, 37, 38]
23.	Jolantidine	*M. jolantae*	[8, 30]
24.	Jolantimine	*M. jolantae*	[8, 30]
25.	Iolantinine	*M. jolantae*	[8, 39]
26.	Trigamine	*M. trigina*	[8, 40]

* photoisomer, which has not been obtained as a natural product but a dimer of β-lumicolchicine

27.	Trigamine-N-oxide	*M. jolantae*	[8]
28.	2-(3,4-Dihydroxyphenyl)-5,7-dihydroxychromene-4-one	*M. caucasica, M. sobolifera, M. kurdica*	[8, 9]
29.	Cornigerine	*M. sobolifera, M.kurdica, M. filifolia*	[8, 34, 35]
30.	Baytopine	*M. kurdica*	[8, 9]

3. TROPOLONE ALKALOIDS

Tropolone alkaloids are a group of compounds that possess a tropolone ring fused with a seven-membered ring in their structures. Colchicine (**1**) is a typical example of this group of compounds.

3.1 DETECTION OF TROPOLONE ALKALOIDS

The detection of tropolone alkaloids in crude extracts of *Merendera* spp. is usually done using colour reactions. Several colour reactions have been developed among which are:

a) Zeisel reaction [10,12,41].

A solution of ferric chloride (1-5%) in HCl (0.5 N) was found to be particularly sensitive to the tropolone nucleus of colchicine alkaloids and amounts as small as 1 µg have been detected. Green colour will be developed after spraying this reagent on a TLC plate.

b) Dragendorff's spray reagent according to Munier.

Orange spots will be observed after spraying on TLC.

c) Marquis test [42]

A yellow colour will be developed (sensitivity: 0.25 µg) when a tropolone alkaloid reacts with formaldehyde solution and H_2SO_4.

d) Liebermann's test [42]

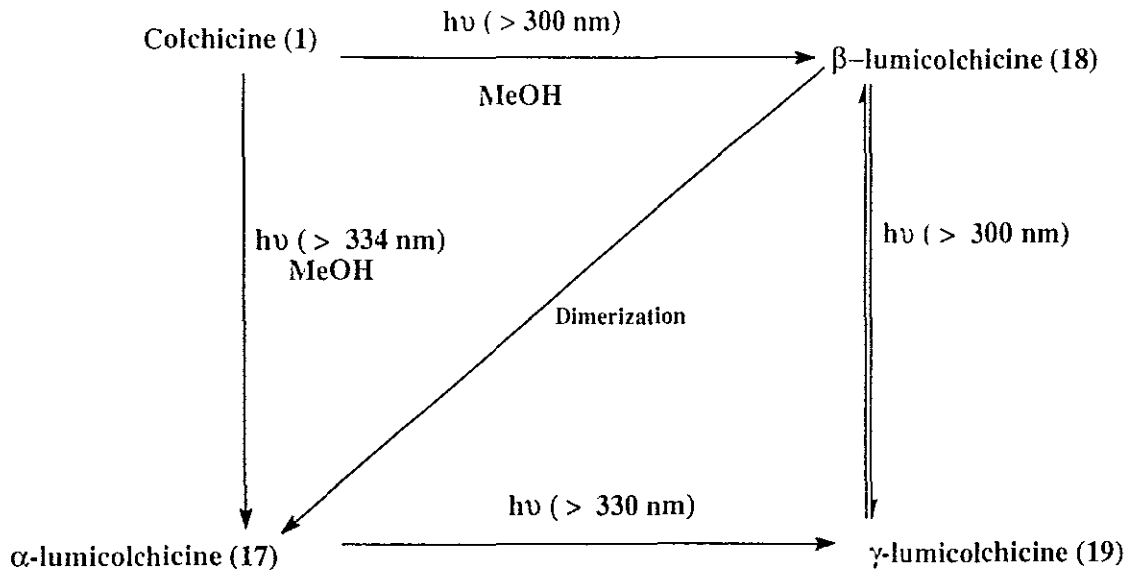
Reaction of sodium nitrite and H_2SO_4 with a tropolone alkaloid results in the development of a green colour (sensitivity: 0.25 µg).

e) Mandelin's test [42].

A green colour will be developed when a dilute solution of ammonium vanadate and H_2SO_4 in water reacts with a tropolone alkaloid.

3.2 PHOTOISOMERIZATION OF TROPOLONE ALKALOIDS

Tropolone alkaloids can be converted into different photoisomers when exposed to ultraviolet light of different wavelengths. For example, colchicine is converted into α-lumicolchicine (**17**), β-lumicolchicine (**18**) and γ-lumicolchicine (**19**). The mechanism of the formation of these compounds involves two disrotatory modes of cyclization of the tropolone ring. Two of them, the β- and the γ-isomers, are known naturally, but α-lumicolchicine (**17**) is a dimer of the β- isomer and yet not found naturally [43]. Scheme 1 shows the photoisomerization of colchicine (**1**) in to the three photoisomers.

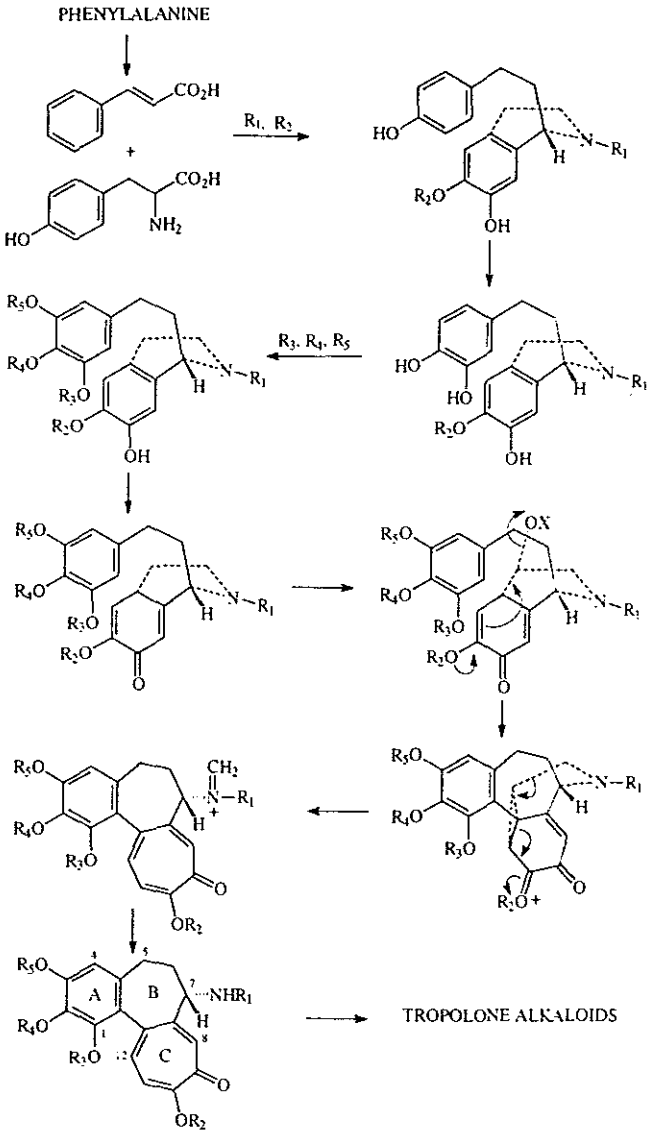


Scheme 1. Photoisomerization of colchicine (**1**) [43].

3.3 BIOSYNTHESIS OF TROPOLONE ALKALOIDS

The biosynthesis of tropolone alkaloids has attracted much attention due to the medicinal importance of colchicine and some of its derivatives [44].

The immediate precursors of tropolone alkaloids are believed to be homoaporphine alkaloids. The homoaporphines originate *via* a metabolic pathway from phenethyltetrahydroisoquinoline precursors [9,45].



Scheme 2. Biosynthesis of tropolone alkaloids [23, 43, 45, 46].

6. RESULTS AND DISCUSSION

Merendera schimperi was collected near Debre Libanos *ca* 110 kms north of Addis Ababa, on the road to Gojjam. The plant was identified by Dr Sebsebe Demissew of the Biology Department, AAU.

Details about the extraction and isolation of compounds from the corm and corm cover of *M. schimperi* are given in the Experimental section. The characterizations of the compounds are discussed below.

6.1 *CHARACTERIZATION OF THE COMPOUNDS*

The dried and powdered corm cover of *M. schimperi* was first extracted with $CHCl_3$ **(Extract-1)** in a Soxhlet apparatus and followed by MeOH extraction **(Extract-2).** The corm of the plant was also extracted with $CHCl_3$ **(Extract-3)** and with MeOH (**Extract-4**) using a Soxhlet apparatus.

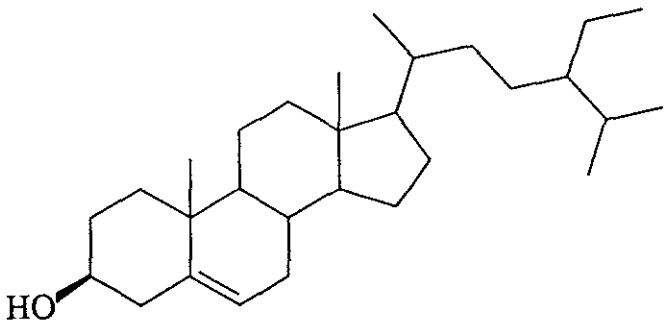
All extracts were subjected to column chromatographic separations on silica gel. The progress of the separation was followed by analytical TLC. Similar fractions were combined and further purified by Prep TLC or by column chromatography on silica gel or by chromatography over Sephadex LH-20. The elucidation of the structures of the isolated compounds were made based on their ^{1}H-NMR, IR and LRMS spectra.

The identification of the compounds is discussed below.

6.1.1 β-*Sitosterol (31)*

Fraction 42-48 of the $CHCl_3$ extract of the corm cover (**Extract 1**) and fraction 9 of the combined $CHCl_3$ and MeOH extracts of the corm (**Extract 3** and **Extract 4**) afforded a white crystalline material (175 mg). The compound was optically active ($[\alpha]_D^{25}$ = -30°(1, $CHCl_3$)) and had a melting point of 148-150° C. This compound was identified as β -sitosterol (**31**) based on the data presented below.

The presence of an -OH was derived from the IR absorption band at 3436 cm-1. The ^{1}H-NMR spectrum displayed a signal at δ5.4 integrating for one proton suggesting the presence of only one olefinic proton. The one proton multiplet at δ 3.5 is assignable to the proton attached to C-3. The aliphatic protons (48 H) resonate in the range between δ0.7 and 2.3 and are unresolved.



31

The IR absorption bands at 2920 cm^{-1} and 2853 cm^{-1} are due to aliphatic C-H stretching.

Comparison of the IR, ^{1}H-NMR, mp and $[\alpha]_D^{25}$ data with those reported in the literature [50] for β-sitosterol showed a good agreement. The compound was also compared by co-TLC with authentic β-sitosterol and the two compounds were found to be identical.

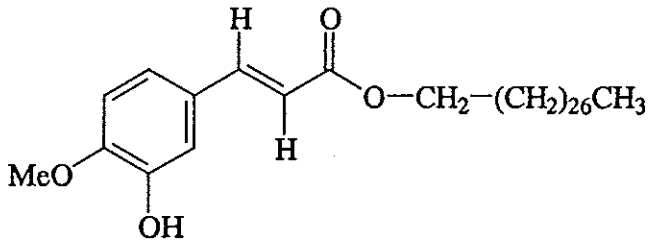
The mixed melting point of the compound with authentic β-sitosterol was undepressed.

6.1.2 *Erythrinasinate (32)*

Fraction 16 (eluted with CH_2Cl_2) of the chromatography of **Extract-1** gave one major spot on TLC (Rf 0.35, pet. ether:EtOAc, 8:2). The melting point of the white crystalline compound (40 mg) was found to be 78-80°C. This compound was identified as erythrinasinate (**32**) as described below.

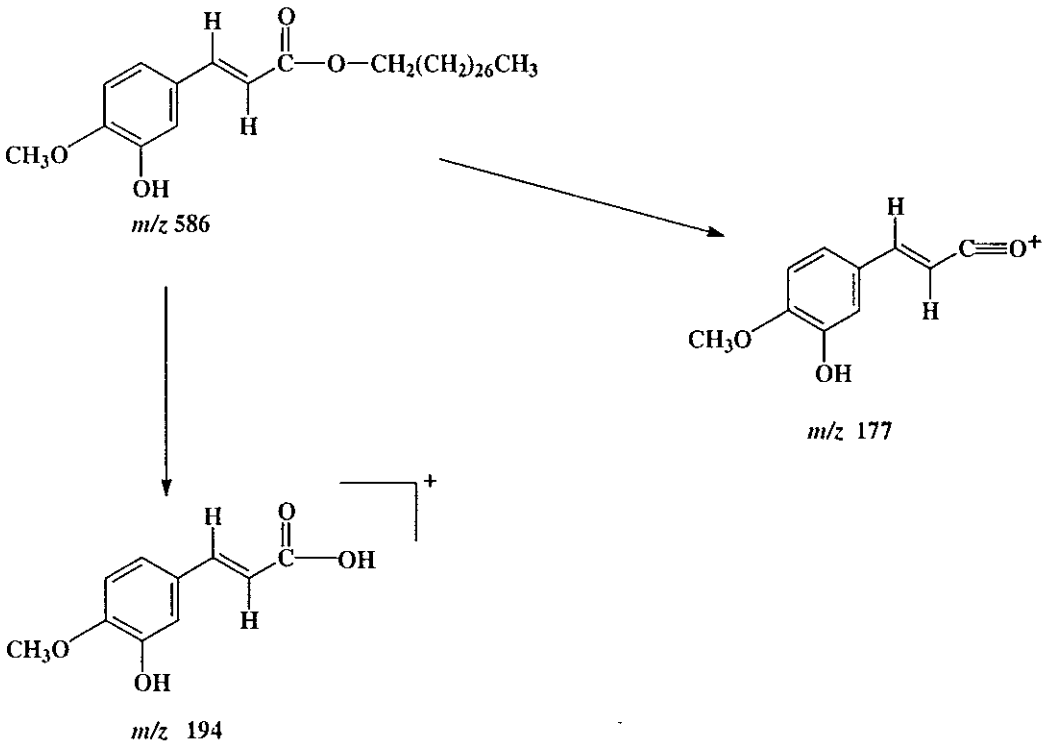
The ^{1}H-NMR spectrum (Figure 1) showed proton resonances at δ6.50 (*d*, *J* = 16.3 Hz) and 7.8 (*d*, *J* = 16.3 Hz). The chemical shifts and the magnitude of the coupling constant suggested the presence of a *trans*-double bond of an α,β-unsaturated carbonyl moiety. Three aromatic proton signals appeared at δ7.15 (*d*), 7.45(*s*) and 7.5(*d*). This suggested the presence of a 1,3,4-trisubstituted aromatic moiety in the molecule. The signal at δ6.10(*s*) is attributable to a phenolic O-H group. The signal at δ1.48, integrating for *ca* 52 protons, suggested the presence of an aliphatic chain of 26 methylene units. In addition the presence of an OCH_3 and an OCH_2- was derived from the resonances at δ4.15 and 4.4, respectively. The LRMS displayed a molecular ion at *m/z* 586 (1.77%)

in agreement with the molecular formula $C_{38}H_{66}O_4$. Major fragment ions appeared at *m/z* 194, 177, and 94. The fragment ions at *m/z* 194 and 177 originate from the molecular ion as shown in the Scheme 3.



32

Comparing the mp 78-80°, (lit.[50], 75-76°), ^{1}H-NMR, and MS data of the compound with those reported in literature [50,51] for erythrinasinate (**32**) confirmed the two compounds to be identical.



Scheme 3. Mass spectral fragmentation pattern of erythrinasinate (**32**).

In addition direct comparison was made by TLC and co-TLC with authentic erythrinasinate and the two compounds were found to be identical.

To our knowledge this is the first finding of erythrinasinate in the genus *Merendera*.

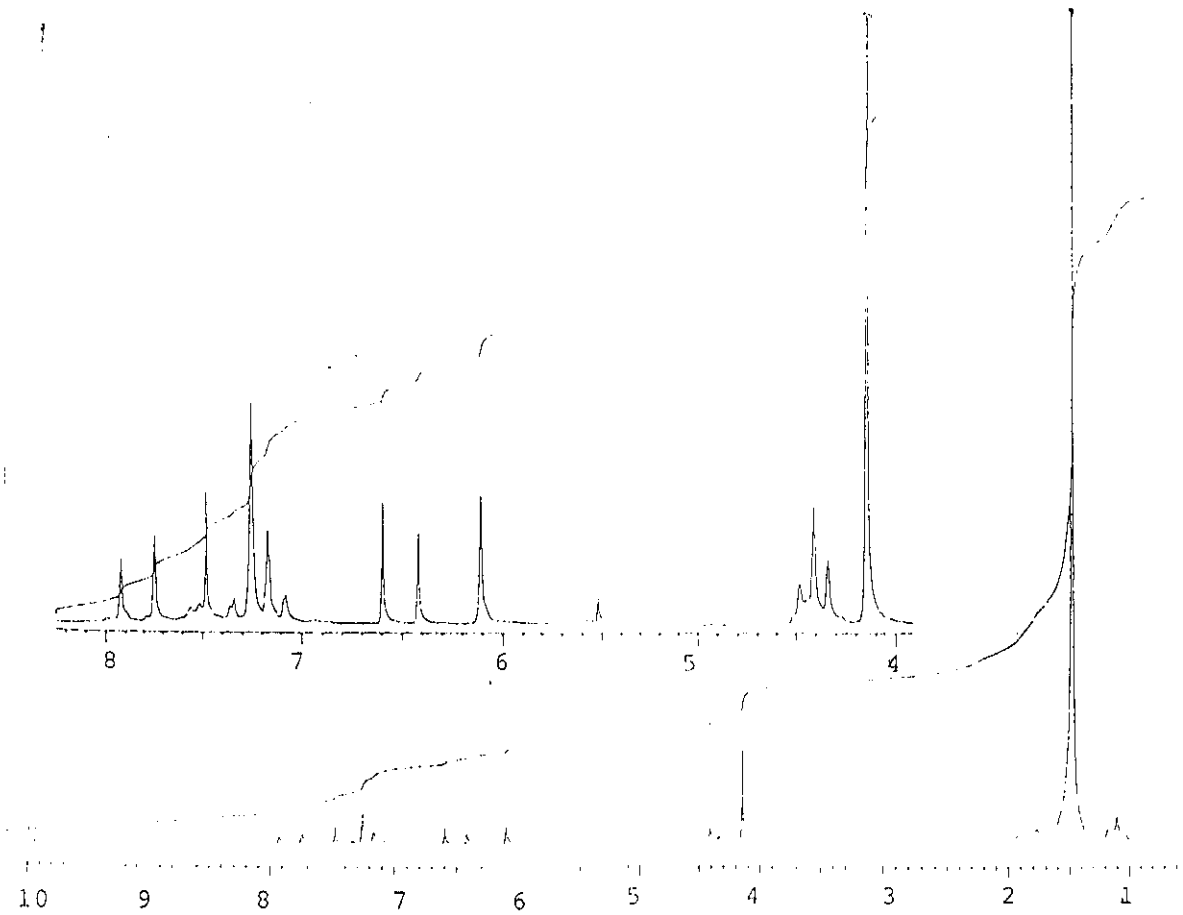
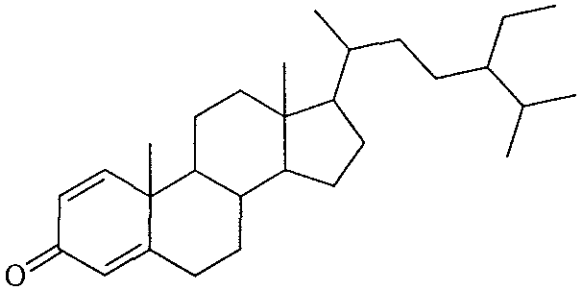


Figure 1. The ^{1}H-NMR spectrum of erythrinasinate **(32).**

6.1.3 *Compound 33*

Compound **33** was obtained as a yellow crystalline material from fractions 54-71 of the chromatography of **Extract-1** eluted with CH_2Cl_2:MeOH (100:1). The ^{1}H-NMR spectrum displayed signals at δ0.7-2.5 (unresolved), 6.05 (*brs*), 6.2 (*dd*) and 7.05 (*d*).

The IR spectrum showed a strong absorption band at 1667 cm^{-1} suggesting the presence of an α,β-unsaturated carbonyl moiety. The lack of a broad absorption band above 3000 cm^{-1} revealed the absence of an OH group. The highest mass ion observed in the LRMS is at *m/z* 410, corresponding to the molecular formula $C_{29}H_{46}O$. Major fragment ions appeared at *m/z* 122 (100%), and 94 (22%). The ^{1}H-NMR spectrum suggested that compound **33** is a triterpene containing two double bonds (see Figure 2).

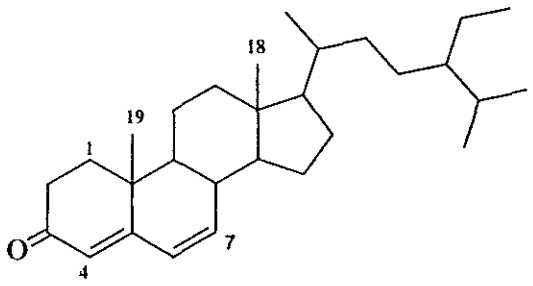


33

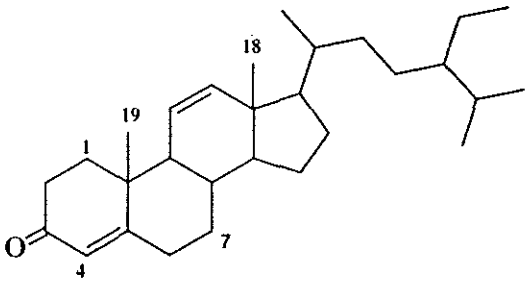
The signal at δ7.05 appears as a doublet (J = 11 Hz) and is coupled with the signal at δ6.2 (*dd*, J = 11, 2.2 Hz). The signal at δ6.05 appears as a broad singlet. The remaining proton resonances appear between δ0.7 and 2.5 and are not well resolved.

It is evident from the spectroscopic data discussed above that compound **33** is a triterpene containing two double bonds and a carbonyl function. Several alternative structures such as structures **33, 33a, 33b** and **33c** can be assigned to this compound. Structure **33** was found to be the most reasonable of all based on the chemical shifts of the olefinic proton signals as discussed below.

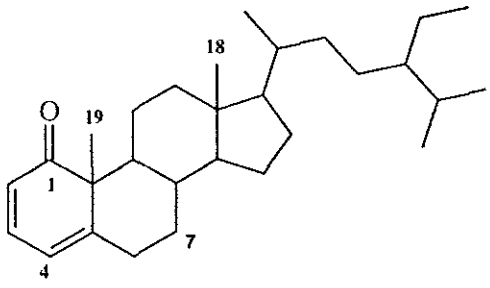
According to structure **33a**, the chemical shift value for the proton at C-7, which is at the δ position relative to the carbonyl group, should be a doublet of doublets and should appear downfield relative to the proton at C-6 (*d*). Hence, structure **33a** can not be a valid alternative. In structure **33b**, the olefinic proton signal due to H-11 should appear as a doublet of doublets and downfield relative to that of H-12. In addition, the chemical shift values are expected to be in the range of δ6.05 to 6.25 [52]. These kinds of signals are not observed in the ^{1}H-NMR



33a



33b



33c

spectrum of the compound. Thus structure **33b** is also an unlikely alternative. If the structure of the compound were **33c**, three olefinic proton signals should have been observed in its ^{1}H-NMR spectrum for H-2, H-3, and H-4 as doublet of doublets. But such signals are not observed in the ^{1}H-NMR spectrum of the compound.

Thus, structure **33** was concluded to be the most reasonable of all the alternatives considered. The proton signals at δ6.05 (*brs*), 6.2 (*dd*) and 7.05 (*d*) can be attributed to H-4, H-2 and H-1, respectively. The proton at C-1 (β-carbon) appears downfield than the protons at C-2 and C-4 (α-carbons). Moreover, the signal due to H-4 appears as a broad singlet at δ6.05 presumably because of long-range coupling with H-2.

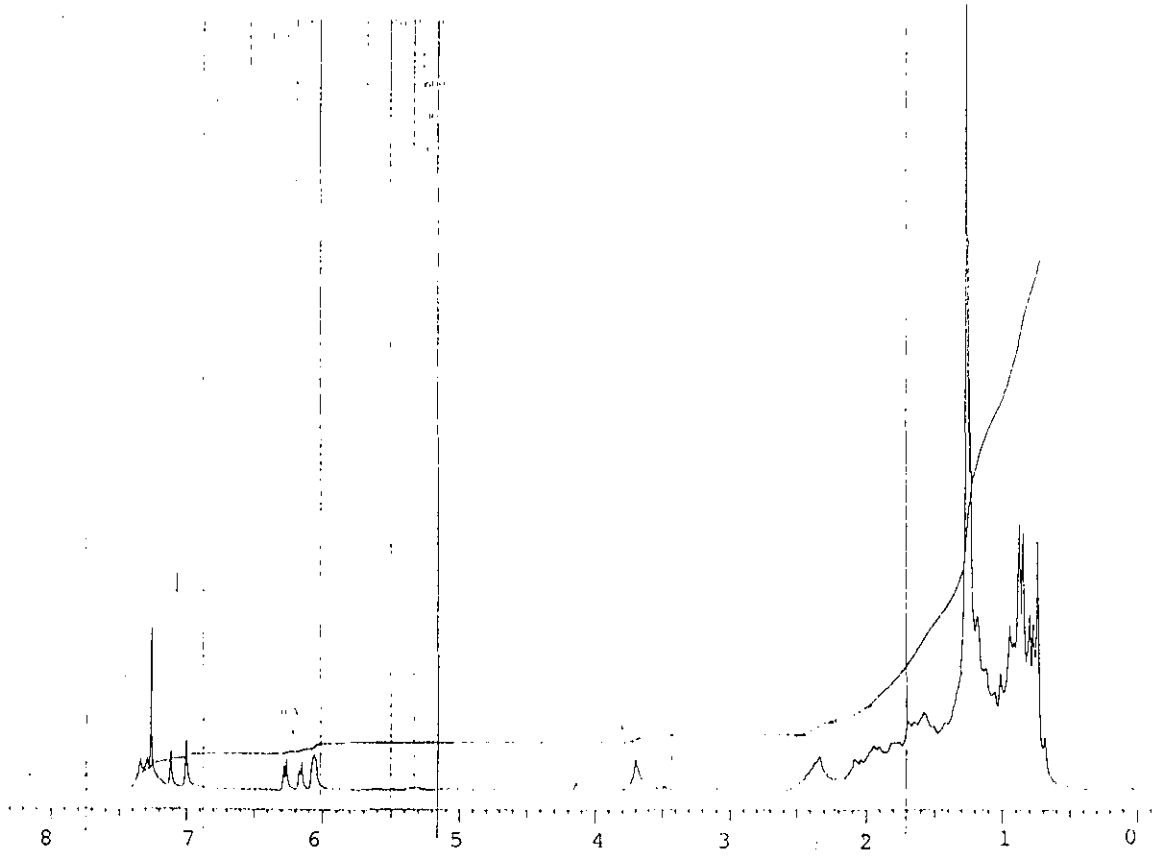
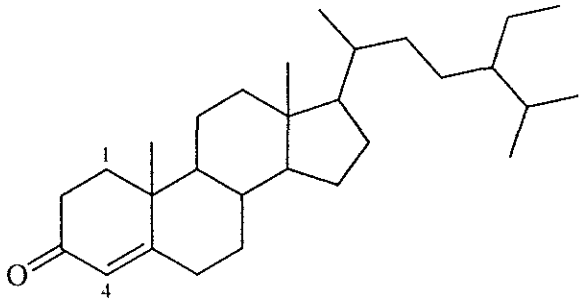


Figure 2. The ^{1}H-NMR spectrum of compound **33.**

6.1.4 *Compound* 34

Compound **34** was also obtained from fractions 54-71 of the chromatography of **Extract-1** eluted with CH_2Cl_2:MeOH (100:1). It was isolated as a yellow crystalline material with mp 72-75° C. The IR spectrum showed a strong absorption band at 1677 cm^{-1} suggesting the presence of an α,β-unsaturated carbonyl group. The absence of an -OH group could be deduced from the lack of a broad band above 3000 cm^{-1}. The ^{1}H-NMR spectrum showed unresolved signals in the region between δ0.65-2.5 integrating for *ca* 47 protons. The broad singlet at δ5.7 revealed the presence of one olefinic proton. It is evident from the ^{1}H-NMR spectrum that this compound is a triterpene .

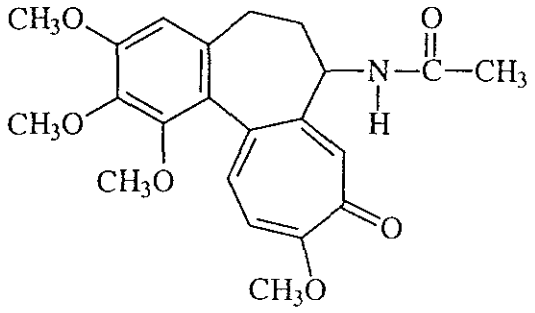
Comparison of the mp, IR and ^{1}H-NMR spectra of this compound with those reported [50,52,53] for stigmast-4-ene-3-one showed a very good agreement. Therefore, compound **34** was identified as stigmast-4-ene-3-one.



34

6.1.5. *Colchicine (1)*

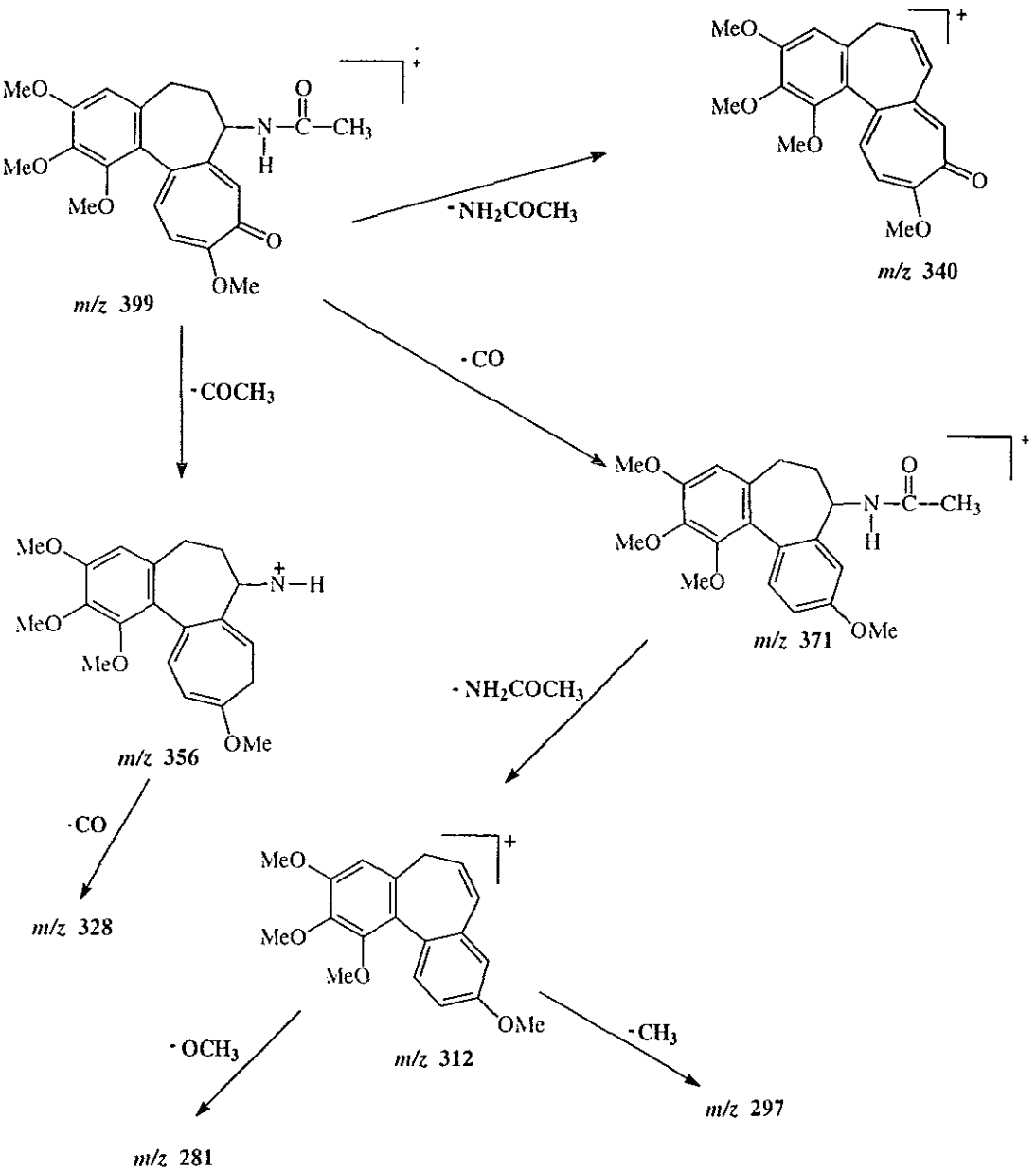
Colchicine (**1**) was obtained from fractions 105-110 of the chromatography of **Extract-1** eluted with CH_2Cl_2:MeOH (100:2), and also from fractions 56-68 of the chromatography of **Extract-2** eluted with EtOAc:MeOH (7:3) and from fractions 90-96 of the chromatography of combined extract of **Extract-3** and **Extract-4** eluted with EtOAc:MeOH (8:2). It was a yellow crystalline material with mp 148-152° (lit.[8], 150-152°), and $[\alpha]_D^{25}$ -120° (c = 1, $CHCl_3$) (lit. [54], -121°, c = 1, $CHCl_3$). It was soluble in chloroform, methanol, ethanol and water. It was positive to Dragendorff's test, which shows that it is an alkaloid. It also gave a green colour with $FeCl_3$ (Zeisel reaction), suggesting that it is a tropolone alkaloid.



1

The IR a strong absorption band at 1590 cm^{-1} is assignable to an amide carbonyl group. The ^{1}H-NMR spectrum (Figure 3) revealed four singlets, integrating for three protons each, at δ3.6, 3.80, 3.90 and 3.95, suggesting the presence of 4 OMe groups. The three proton singlet at δ2.0 is attributable to an acetyl methyl group. Three olefinic proton resonances appeared at δ7.6 (*s*, 1H), 6.86 (1H, *d*,*J* =11 Hz) and δ7.42 (1H, *d*, *J* = 11 Hz), and are assignable to H-8, H-11 and H-12, respectively. The singlet at δ6.5 can be assigned to the aromatic proton at C-4.

The remaining proton signals appeared at δ4.50 (1H, *m*, H-7), 7.95 (1H, *d*, N-H), 2.4 (*m*, 2H) and 1.9-2.1 (unresolved, 2H). The LRMS displayed a molecular ion at *m/z* 399(100%) in agreement with the molecular formula $C_{22}H_{25}NO_6$. Major fragment ions appeared at *m/z* 371 (37%), 356 (16%), 340 (12%), 328 (8%), 312 (97%), 297 (30%) and 281 (23%). The origin of some of the important ions from the molecular ion is shown in Scheme 4.



Scheme 4. Mass spectral fragmentation pattern of colchicine (**1**).

Comparison of the IR, ^{1}H-NMR, MS, mp and $[\alpha]_D^{25}$ data with those reported in the literature [8, 54, 55] for colchicine, showed a very good agreement.

Direct comparison of the ^{1}H-NMR and TLC of our compound was also made with authentic colchicine obtained from the Drug Research Department (Ethiopian Health and Nutrition Research Institute, EHNRI). The two compounds showed identical properties.

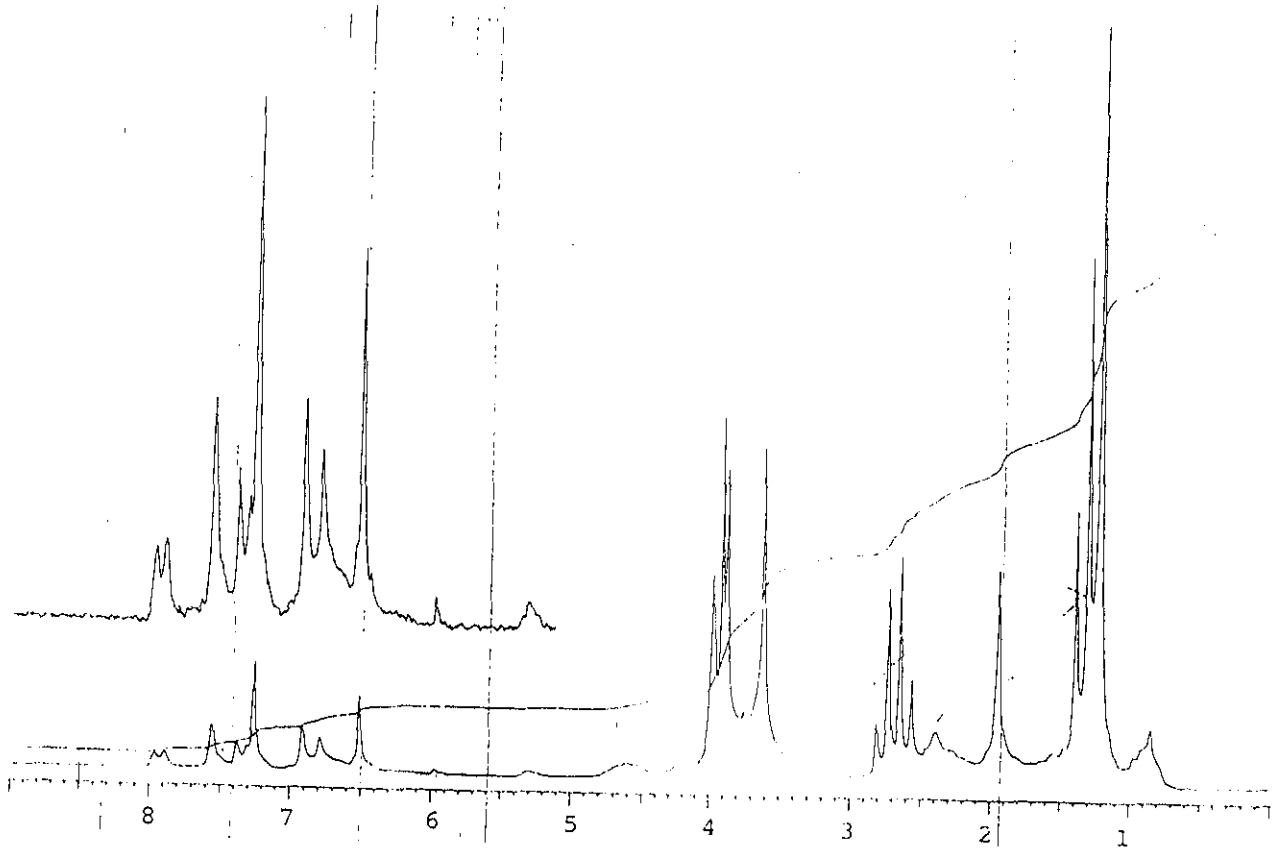
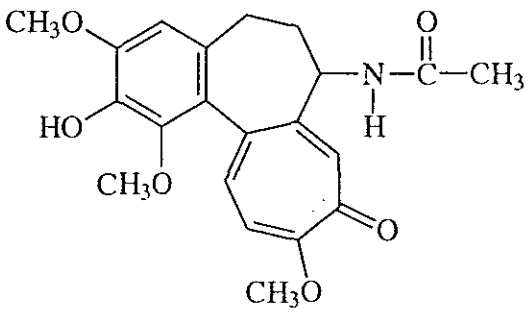


Figure 3. The ^{1}H-NMR spectrum of colchicine **(1).**

6.1.7 *2-Demethylcolchicine* (2)

Colchicine was converted to 2-demethylcolchicine (**2**) according to the method of Rosner *et. al.* [54] (see the Experimental section). The synthesis was attempted in order to find out whether the extracts of the plant contain demethylated colchicine compounds or not. Compound **2** was obtained, after purification by prep. TLC, as a yellow crystalline material with mp 176-78° (lit. [54], 176-82°). The ^{1}H-NMR spectrum showed a singlet at δ1.95 (3H, *s*) due to an acetyl methyl group. The signals due to the CH_2 protons attached to C-5 and C-6 appeared at δ1.75 and 2.4 and were not well resolved. Three OMe resonances appeared at δ3.6 (*s*), 3.90(*s*) and 3.95(*s*). The proton resonance at δ4.6 (1H, *m*) was due to H-7. The doublets at δ6.85 (J = 11 Hz) and 7.45 (J = 11 Hz) and the singlets at δ6.5 and 7.55 are attributable to the olefinic and aromatic protons at C-11, C-12, C-4 and C-8, respectively (see Figure 4). Comparison of the IR, ^{1}H-NMR and mp with those reported for 2-demethylcolchicine in the literature [54] revealed the two compounds to be identical. Unfortunately this compound was not detected in any of the extracts of *M. schimperi.*



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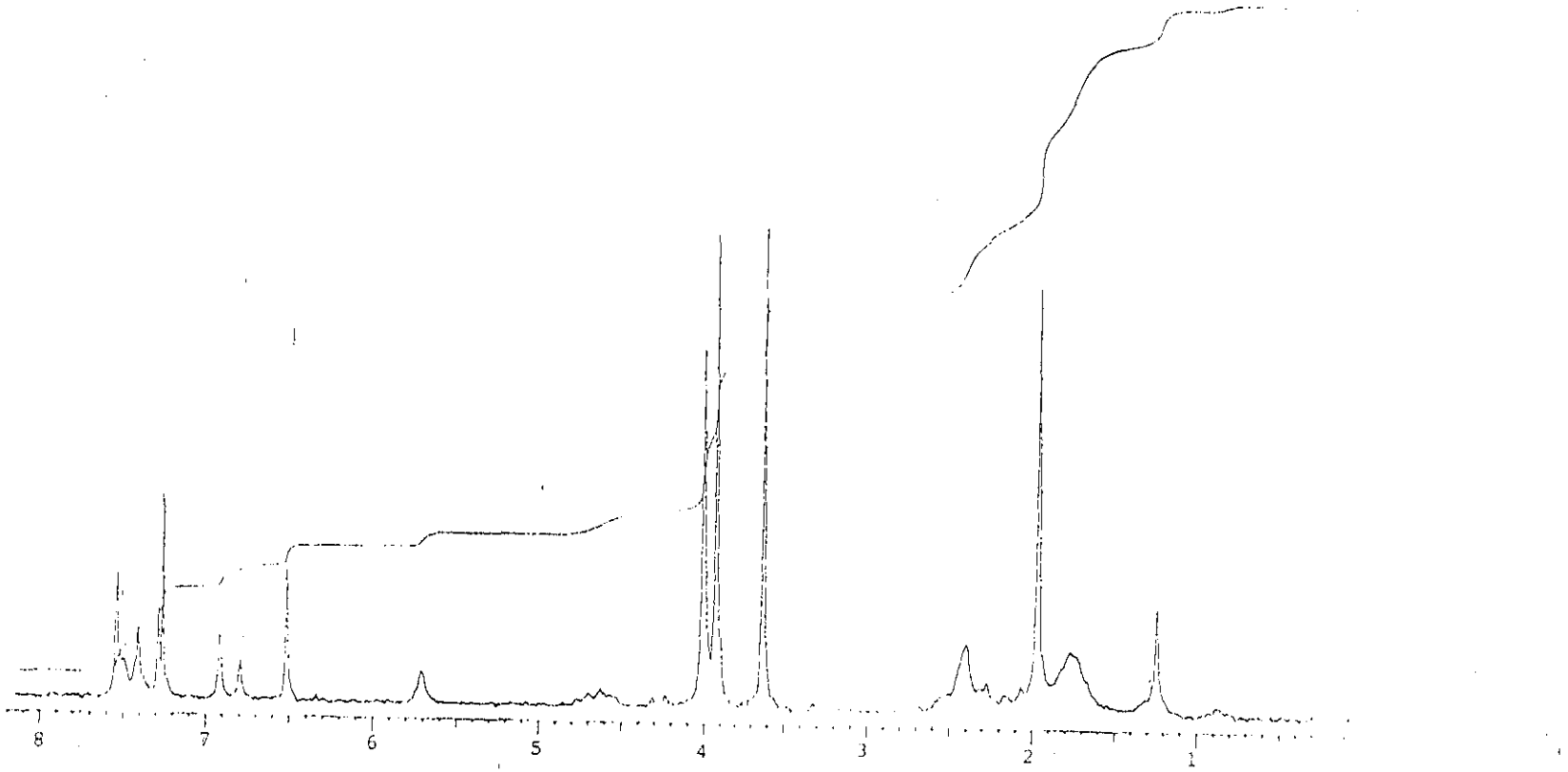
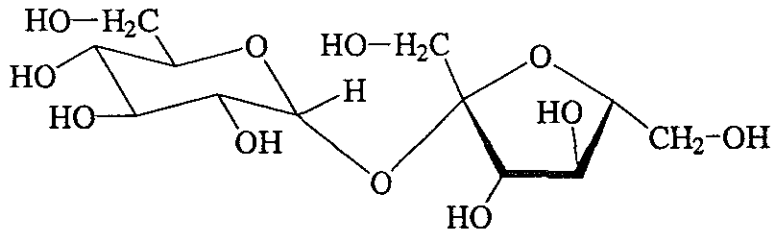


Figure 4. The ^{1}H-NMR of 2-demethylcolchicine **(2).**

6.1.8 *Compound* 35

Compound **35** (125 mg) was obtained from fraction 124 of the chromatography of the combined extract of **Extract-3** and **Extract-4**. It was isolated as a white crystalline material with mp 182-184°, (lit.[56] 185-187°), $[\alpha]_D^{25}$ +70° (c = 1, D_2O, lit [56], +66.47°, c = 26, H_2O). It was soluble in H_2O and EtOH and insoluble in $CHCl_3$. The ^{1}H-NMR was recorded in D_2O solution and showed multiplets in the region between δ3.15 and 4.1 (13 H), and a doublet at δ5.20 (1 H). The ^{1}H-NMR spectrum suggested that this compound is a disaccharide. Comparison of the ^{1}H-NMR spectrum of this compound with a standard sucrose spectrum revealed that the two compounds are identical. This was further confirmed by paper chromatography of compound **35** with standard sucrose and by developing the paper using a spray reagent prepared according to literature [57]. Thus compound **35** was identified as sucrose.

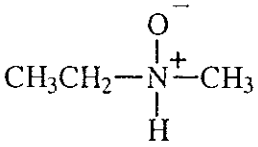


35

6.1.9 *Compound 36*

Compound **36** was obtained from fractions 78-86 of the chromatography of the combined extracts of **Extract-3** and **Extract-4** eluted with EtOAc:MeOH (8:2). It was a yellow crystalline material (mp 42-45°C).

The ^{1}H-NMR spectrum showed signals at δ1.25 (3H, *t*), 2.2 (1H, *b*), 3.4 (3H, *s*), 3.7 (2H, *q*) (see Figure 5). The signals at δ1.25 and 3.7 reveal the presence of an ethyl group while the signal at δ3.4 is attributable to an N-CH_3 group. The presence of N-H was derived from the broad absorption band in the IR spectrum at 3444 cm^{-1}. The chemical shifts of the methylene (δ3.7) and methyl (δ3.4) groups suggest that the nitrogen atom is positively charged and that the compound is presumably an ammonium salt. Comparison of the melting point of compound **36** with that of ethylmethylamine hydrochloride (mp 126-130) revealed a very big difference. Thus, this compound can be assigned structure **36**.



36

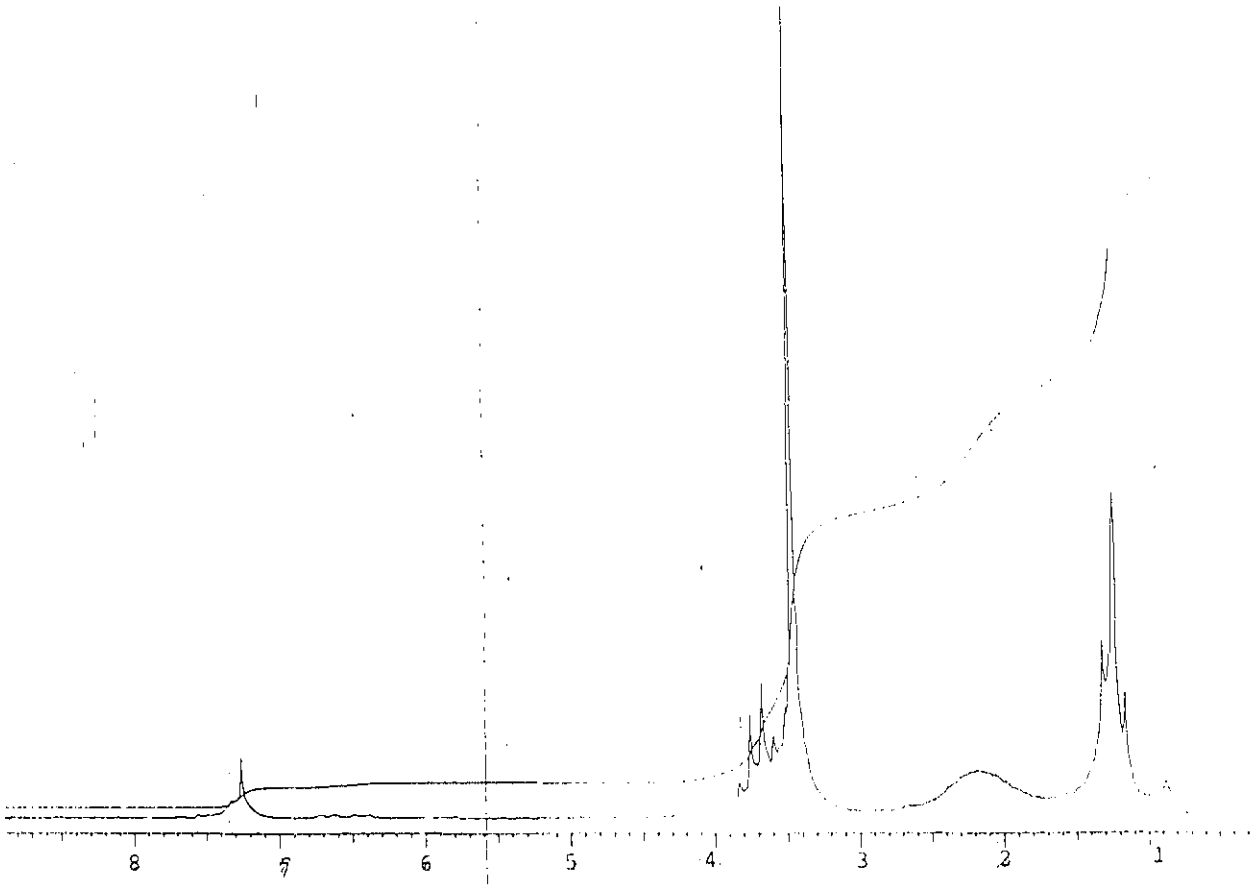


Figure 5. The ^{1}H-NMR of Compound **36.**

7. RECOMMENDATIONS

The use of *Merendera schimperi* in the traditional medicine of Ethiopia has not been well documented. The related species *Glorioisa superba* ("**Etse libona**"), is, however, used in the treatment of scorpion bite [49]. This study showed that the major alkaloid in *M. schimeperi* is colchicine. Colchicine is an important compound that has been used in the treatment of acute gout. We believe that *M. schimperi* can find an important application in the treatment of gout, particularly in the countryside, where modern medicine is unavailable and unaffordable. It is however important to conduct a laboratory study to determine the dosage and mode of administration prior to recommending the wide use of the plant by the population. It is to be noted that colchicine has an LD_{50} of 7 mg [58]. This makes the compound quite toxic.

Furthermore, colchcine obtained from different sources of the genus *Merendera*, has shown activities against different kinds of diseases [9, 10]. This suggests that *M. schimperi* can also find other kinds of applications other than the treatment of gout.

Botanists claim that the only *Merendera* species that grows in Ethiopia is *M. schimperi* . As has been mentioned above *Gloriosa superba* is the only related plant growing in Ethiopia. It will therefore be very appropriate to conduct a comparative study of the chemistry of these two plants. Such a study will have a chemotaxonomic significance.

8. EXPERIMENTAL

INSTRUMENTS

IR: Perkin -Elmer 1600 series, FTIR

^{1}H-NMR: Joel FX-90Q (90 MHz); Values are given in ppm; Multiplicities: *s* =singlet *d* =doublet, *t* = triplet; *m* = multiplet, *q* = quartet, *b* = broad.

LRMS: Faden, MAT-95Q (El+Ve)

Optical rotation: Perkin-Elmer 41 polarimeter

MP: Kofler block melting point apparatus and are uncorrected

Chromatography

Analytical TLC: Silica gel 60 F_{254} (Merck) and Silica gel (Fluka)

Preparative TLC:

1. Silica gel 60 $F_{254+366}$ (Merck)
2. Silica gel GF_{254} (Fluka)

Column chromatography: Silica gel 60 (Merck).

Spray reagents:

1) Dragendorff's reagent was prepared according to Munier.
2) Oberlin Zeisel reaction was conducted to detect tropolone alkaloids [10].
3) Reagent for detection of sugars was prepared according to Kamusiime [57]. Thus, diphenylamine (2 g), aniline (2 ml), acetone (100 ml) and phosphoric

acid (15 ml) were mixed just before sparying the TLC plates.

8.1 *PLANT MATERIAL*

Merendera schimperi was collected from Debre Libanos (North Shoa), 110 km North of Addis Ababa, at an altitude of 2520 m above sea level. The plant was identified by Dr Sebsebe Demissew of the National Herbarium, Biology Department, AAU, and a specimen was deposited at the National Herbarium, Addis Ababa University (Voucher number: Belete Tegbaru *sn*).

8.2. *EXTRACTION*

8.2.1 *Corm cover of M. schimperi*

Coarsely ground corm cover (230 g) of the plant was extracted in Soxhlet apparatus successively for 12 h with $CHCl_3$ (**Extract 1**) and MeOH (**Extract 2).** The extracts were concentrated to yield 7.71 g (3.35%) and 6.90 g (3.0%), respectively, of light yellow gummy material.

8.2.2 *Corm of M. schimperi*

Finely ground corm (125 g) of the plant was extracted with $CHCl_3$ **(Extract 3)** and with MeOH (**Extract 4**) in a Soxhlet apparatus. Removal of the solvents gave 2.2 g (1.76%), and 8.63 (6.94%), respectively, of a gummy residue.

8.3 ISOLATION OF COMPOUNDS

8.3.1 *Extract 1*

7.71 g of **Extract 1** was adsorbed on about 10 g of silica gel. It was chromatographed on a column of Silica gel and eluted with CH_2Cl_2 and CH_2Cl_2:MeOH mixtures of increasing polarity.

A total of 200 fractions, each of *ca.* 50 mL, were collected.

Fraction	Eluent
1-34	CH_2Cl_2
35-56	100:1(CH_2Cl_2:MeOH)
57-76	100:2 >>
77-98	100:5 >>
99-105	10:1 >>
106-130	10:2 >>
131-155	10:4 >>
156-200	1:1 >>

Fraction 16, eluted with CH_2Cl_2, was further purified by prep TLC (Pet. ether:EtOAc, 8:2) on 1 mm Silica gel plates. The major band (Rf 0.35) was scrapped off the plate and gave erythrinasinate (**32**).

Fractions 42-48 were combined and applied on to 1 mm silica gel plates and eluted with CH_2Cl_2: MeOH (100:1). The major band (Rf 0.56, pet.ether:EtOAc, 8:2,) was scrapped off the plate and afforded β-sitosterol (**31**).

Fractions 54-71 eluted with CH_2Cl_2:MeOH (100:2) were combined and further purified by prep. TLC. The compounds with Rf 0.34 and 0.49 (Solvent: pet ether: EtOAc, 9:1) were scrapped off the plate and labelled as compound **33** and **34.**

Fractions 105-110 eluted with CH_2Cl_2:MeOH (10:2) gave colchicine (**1**, 130 mg) after purification by prep. TLC on silica gel plates using EtOAc:MeOH:H_2O (77:13:10) as eluent.

8.3.2 Extract 2

8.63 g of Extract 2 was adsorbed on silica gel and applied on to a silica gel column. Elution was carried out as shown below. A total of 81 fractions, *ca* 100 mL of each, were collected.

Fraction	Eluent
1-2	Pet.ether
3-4	9:1 (Pet.ether:EtOAc)
5-9	8:2 >>
10-12	7:3 >>
13-16	6:4 >>

17-20	1:1 >>
21-24	6:4 (EtOAc:Pet.ether)
25-27	7:3 >>
28-33	8:2 >>
34-39	EtOAc
40-43	9:1 (EtOAc:MeOH)
44-55	8:2 >>
56-70	7:3 >>
71-81	6:4 >>

Fraction 6, eluted with pet.ether:EtOAc (8:2), afforded compound **34** in a pure form.

Fractions 56-68, eluted with EtOAc:MeOH (7:3), contained one major compound which gave positive reactions to Dragendorff's reagent (yellow) and Oberlin-Zeisel reagent (green colour). The major compound (Rf 0.42) was obtained in pure form after prep. TLC on 1 mm silica gel plates using EtOAc:MeOH:H_2O (77:13:10) as eluent. The compound was identified as colchicine (**1**).

8.3.3 *Extracts 3 and 4*

Extracts 3 and **4** were combined, because they had similar TLCs. 10.83 g of the mixture was adsorbed on about 20 g of silica gel and was chromatographed on a silica gel Column using pet. ether, pet.ether:EtOAc and EtOAc:MeOH mixtures of increasing polarity. A total of 136 fractions, *ca* 50 mL each, were collected.

Fractions	Eluent
1-5	9:1 (Pet.ether:EtOAc)
6-10	8:2 >>
11-17	7:3 >>
18-22	1:1 >>
23-41	6:4 (EtOAc:Pet.ether)
42-51	7:3 >>
52-56	8:2 >>
57-72	EtOAc
73-77	9:1 (EtOAc:MeOH)
78-99	8:2 >>
100-118	7:3 >>
119-136	6:4 >>

Fraction 9 contained one major compound. The compound was identified as β -sitosterol (**31**) by comparing with an authentic sample of β-sitosterol.

Fractions 78-86 contained a mixture of compounds. The mixture was applied on to 1 mm silica gel plates and the fastest moving band (Rf 0.60, $CHCl_3$:EtOAc, 8:2), which showed a blue fluorescence on the 366 nm UV light, was scrapped off the plate. It was labelled as compound **36**.

Fractions 90-96 were combined and on to 1 mm silica gel. The major band (Rf 0.46, EtOAc:MeOH:H_2O, 77:13:10) was scrapped off the plate and was identified as colchicine (**1**) by direct comparison with an authentic sample of colchicine.

Fraction 124 was taken up in MeOH. The MeOH insoluble portion afforded compound **35** which was identified as sucrose..

8.4 Synthesis of 2-demethylcolchicine (2) from Colchicine (1).

The method of Rosner *et al.* [54] was adopted in the preparation of 2-demethylcolchicine (**2**) from colchicine (**1**) (Commercial grade, obtained from EHNRI).

A mixture of 50 mg of colchicine and 250 µl of conc. H_2SO_4 was stirred at 60 ± 2°C for 5 h. The progress of the reaction was monitored by TLC using CH_2Cl_2:MeOH (9:1). The resulting yellow solution was poured on to ice and adjusted to pH 5 with 2N NaOH. The solution was extracted with 300 mL of CH_2Cl_2. The extract was dried using Na_2SO_4, filtered and evaporated to afforded 15 mg of the product. Examining the final product by TLC (CH_2Cl_2:MeOH (95:5)) revealed that there was trace of unreacted colchicine. Therefore, the product was purified by prep. TLC using CH_2Cl_2:MeOH (95:5) as eluent. The band with Rf 0.20 was scrapped off the plate and was identified as 2-demethylcolchicine (**2**).

8.5 β-Sitosterol (31)

White crystalline compound, mp 148-150°; $[\alpha]_D^{25}$ = -30° (c = 1, $CHCl_3$); IR υ_{max} cm^{-1}: 3436, 2920, 2853, 1622, 1458, 1368, 1056, 957; ^{1}H-NMR (90 MHz, $CDCl_3$: δ 0.6-2.3 (48 H, unresolved), 3.5 (1H, *m*, 3-H), 5.35 (1H, *bd*, 6-H).

8.6 *Erythrinasinate (32)*

White crystals, mp 78-80° (lit. [51], 75-76°). ^{1}H-NMR (90 MHz, $CDCl_3$): δ1.1 (3H, *t*, Me-27'), 1.48 (52H, *s*, $(CH_2)_{26}$), 4.15 (3H, *s*, OCH_3), 4.4 (2 H, *t*, O-$\underline{CH_2}$-CH_2-R), 6.10 (1H, *s*, O-H), 6.50 (1H, *d*, *J*=16.3 Hz, =C-H), 7.15 (1H, *d*, H-6), 7.45 (1H, *s*, H-2), 7.5 (1H, *d*, H-5), 7.8 (1H, *d*, *J*=16.3 Hz, =C-H). IR υ_{max}cm^{-1}: 3436, 2921, 2850, 1715, 1591, 1462, 1358, 1274, 1148, 1026, 857. LRMS *m/z* (rel. int): 586 $[M]^+$ (6), 194 (13), 177 (27), 94 (100).

8.7 *Compound 33*

Yellow crystals. mp 68-71°, ^{1}H-NMR (90 MHz, $CDCl_3$): δ0.7-2.5 (unresolved), 6.05 (*bs*), 6.2 (*dd*, *J* = 11.7, *ca* 2.2 Hz,), 7.05 (*d*, *J* = 11.7 Hz); IR υ_{max} cm^{-1}: 2929, 2855, 1667, 1458, 1376 1069; LRMS *m/z* (rel. int.): 410 $[M]^+$ (7.27), 122 (100), 94(22).

8.8 *Compound 34*

Yellow crystals, mp 72-75° (lit.[53] 77-80°); ^{1}H-NMR (90 MHz, $CDCl_3$): δ6.5-2.5 (unresolved), 5.7 (*bs*); IR υ_{max}cm^{-1}: 2933, 2870, 1730, 1677, 1618, 1465, 1435, 1072.

8.9 Colchicine (1)

Yellow crystals, mp 148-152° (lit.[8] 150-152); $[\alpha]_D^{25}$ -120 (c = 1, $CHCl_3$) (lit. [54], -121°, c = 1, $CHCl_3$); IR υ_{max} cm^{-1} : 3394, 2924, 2852, 1590, 1547, 1459, 1352, 1256, 1140, 1090, 857, 605; ^{1}H-NMR (90 MHz, $CDCl_3$): δ2.0 (3H, *s*, CH_3CO), 2.4 (4H, *b*, 2 x CH_2, H-5 and H-6), 3.6 (3H, *s*, 1-OMe), 3.80 (3H, *s*, 2-OMe), 3.90 (3H, *s*, 3-OMe), 3.95 (3H, *s*, 10-OMe), 4.50 (1H, *m*, H-7), 6.50 (1H, *s*, H-4), 6.86 (1H, *d*, *J* = 11 Hz, H-11), 7.42 (1H, *d*, *J* = 11 Hz, H-12), 7.58 (1H, *s*, H-8), 7.95 (1H, *d*, N-H); LRMS m/z (rel. int.): 399 $[M]^+$ (100), 371 (37), 356 (16), 340 (12), 328 (8), 312 (97), 297 (30), 281 (23).

8.10 2-Demethylcolchicine (2)

Yellow crystals. mp 176-78° (lit.[54] 176-82°); IR υ_{max} cm^{-1}: 3430, 2931, 1660, 1590, 1437, 1252, 1179, 1137, 1079, 908; ^{1}H-NMR (90 MHz, $CDCl_3$): δ1.9 (3H, *s*, $COCH_3$), 1.75 (2H, *b*, 6-CH_2), 2.4 (2H, *b*, 5-CH_2), 3.6 (3H, *s*, 1-OMe), 3.90 (3H, s, 3-OMe), 3.95 (3H, s, 10-OMe), 4.6 (1H, m, H-7), 6.6 (1H, *s*, H-4), 6.85 (1H, *d*, *J* = 11 Hz, H-12), 7.45 (1H, *d*, *J* = 11 Hz, H-12), 7.6 (1H, *s*, H-8).

8.11 Compound 35

White crystals, mp 182-184°; (lit.[56] 185-187°), $[\alpha]_D^{25}$ = + 70° (c = 1, D_2O); ^{1}H-NMR (90 MHz, $CDCl_3$): δ3.15 -4.1(unresolved, 13 H), 5.21 (*d*, 1H).

8.12 Compound 36

Yellow crystals. mp 42 - 44°; ^{1}H-NMR (90 MHz, $CDCl_3$): δ1.2 (3H, *t*), 2.2 (1H, *b*), 3.4 (3H, *s*), 3.7 (2H, *q*). IR υ_{max} cm^{-1}: 3444, 2922, 2851, 1591, 1464, 1384, 1240, 1085.

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