

Sesquiterpene lactones from *Inula confertiflora* A. Rich (Asteraceae) Leaf

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Abstract: This study was aimed at the isolation of compounds present in the methanol extract of the leaves of the Ethiopian endemic plant *Inula confertiflora* A. Rich (Asteraceae). Phytochemical study on this extract led to first-time isolation and structural elucidations of reportedly bioactive sesquiterpene lactones, namely 4 α , 5 α -epoxyinuviscolide, graveolide, and carabrone, using spectroscopic methods.

Keywords: *Inula confertiflora*; 4 α ,5 α -epoxyinuviscolide; graveolide; carabrone; sesquiterpene lactones.

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1. Introduction

The genus *Inula*, belonging to the Asteraceae family, is composed of over 100 species distributed in Europe, Asia, and Africa. They are mostly herbs with alternate leaves, sometimes with an offensive smell. Most of the *Inula* species available worldwide are described for their traditional applications and chemical compositions. *I. helenium*, *I. racemosa*, *I. hupehensis*, *I. britannica* and *I. britannica* var. *chinensis* were commonly studied and reported for their bioactivities. More than 400 compounds were isolated from the species studied, of which sesquiterpene lactones are dominant [1-7]. They showed a diversity of chemical structures grouped under eudesmanolides (1), guaianolides (2/3), pseudoguaianolides (4/5), germacranolides (6) and xanthanolides (7) (Fig. 1). Sesquiterpene lactones of these groups displayed a wide range of biological properties including antifungal, anticancer, anti-inflammatory, and antibacterial activities [2-7]. They are the causes of the bitter taste of the plant.

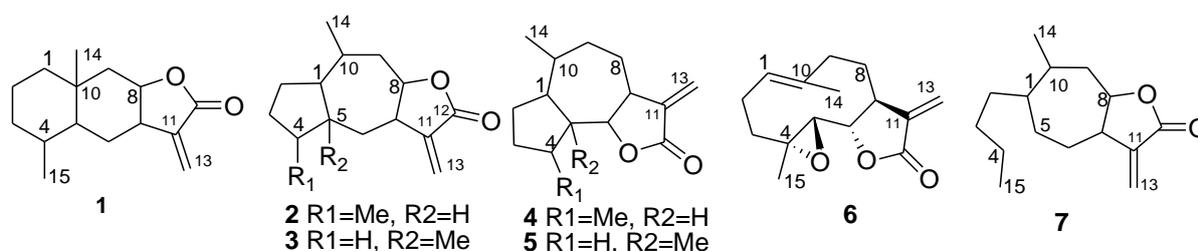


Figure 1. Structures of representative classes of sesquiterpenes lactones.

Inula confertiflora A. Rich is an endemic plant in Shewa, Wollo, Gojam, and Gonder (Ethiopia). An ethnopharmacological study of the plant indicated that *I. confertiflora* is found

applicable to treat skin diseases caused by viruses, wounds, and eczematous lesions. In Ethiopian folklore, dried roots of *I. confertiflora* are smoked as a fumigant during childbirth. The maceration of pounded leaves in water is also applied to the diseased eyes of cattle. Traditional healers in Ethiopia used the roots and flower of the plant against leprosy and the leaf for treating asthma, the common cold, and cough [8]. Although the plant species in the *Inula* genus are rich sources of bioactive sesquiterpene lactones, no prior phytochemical study reports are found on this plant except for antiviral activity studies elsewhere [9-11]. Therefore, this study focused on the isolation of sesquiterpene lactones present in methanol extract of the leaves of *Inula confertiflora*.

2. Materials and Methods

2.1. Chemicals and apparatus.

Methanol, chloroform, ethyl acetate and petroleum ether (n-hexane), ferric chloride, lead acetate, sodium hydroxide, hydrochloric acid, sulfuric acid, glacial acetic acid (analytical grade), and silica gel (60-120 mesh size) were used in this study. Apparatus such as digital analytical balance, electric grinder, rotary evaporator, chromatographic tools, UV light, and NMR-spectrophotometer (Bruker ACQ 400 AVANCE spectrometer operating at 400 MHz) were used. The IR spectra were recorded using a Perkin-Elmer BX Spectrometer (400-4000 cm^{-1}) in KBr.

2.2. Collection and identification of the plant.

Inula confertiflora (Asteraceae, Fig. 2), locally named *Weynagift* (*Amh*), was collected from Ambassel district, 09 kebele, which is located 46 Km north of Dessie town at an altitude of 2757 m above sea level and identification was made by professional botanist Mr. Belay Melese (Assist. Prof.) at Wollo University.



Figure 2. The aerial part of *Inula confertiflora* (Photo by MG).

2.3. Extraction and phytochemical analysis.

Shade dried and ground leaves (100 g) were extracted with petroleum ether, ethyl acetate, and methanol (500 mL) separately by maceration (48 h) at room temperature and concentrated under vacuum using a rotary-evaporator to get the respective crude extracts. The methanol extract was subjected to phytochemical analysis using standard procedures listed elsewhere [12] (Table 1).

Table 1. Phytochemical screening tests for the extracts.

Secondary metabolites	Procedures followed	Expected positive result
Alkaloids	50 mg crude extract dissolved well with 2 mL of 1% HCl solution, followed by slight heating till the steaming. Then six drops of Wagner's reagent was added to 1 mL acidified extract.	The brown precipitate (ppt)
Cardiac glycosides	0.5 g of the extract dissolved into 0.4 mL of glacial acetic acid with some ferric chloride, and then 0.5 ml of conc. H ₂ SO ₄ was added via the side of the test tube	The appearance of blue color in the acid layer
Flavonoids	1 mL of extract added to 1 mL of 10 % lead acetate	yellow ppt
Phenols	1 mL extract added into 0.5 mL of 5 % ethanolic ferric chloride solution.	Dark color formed
Tannins	A few drops of 10 % lead acetate added into 2 mL of extract	Precipitate formation
Terpenoids	2.5 mL of extract mixed with 1 mL of chloroform and then 1.5 mL of conc. H ₂ SO ₄ solution added	Reddish brown color
Anthraquinones test 11. Anthraquinones	1 mL of extract placed on a test tube, and then 5 drops of 2% hydrochloric acid added	The appearance of the red color
Phlobatannin test	10 drops of 1% hydrochloric acid added to 1ml of the crude extract in test tube.	Formation of red precipitate
Phytosterols	50 mg of crude extract dissolved in 2 mL of acetic anhydride, then two drops of conc. H ₂ SO ₄ added	The dark brown color formed
Saponin	50 mg of the extract diluted with distilled water up to 20 mL with strong agitation.	Foam persists for 15 min.

2.4. Isolation and characterization of compounds.

Based on the qualitative TLC analysis of the extract, 10 g of the methanol extract of the leaves adsorbed on silica gel was chromatographed on a silica gel column packed with silica gel (100 g, 60-120 mesh size) with elution gradient, *n*-hexane/ethyl acetate/methanol and 80 fractions (50 mL) were collected (Fig 3). Fractions 35-48 (80:20, hexane/ethyl acetate) were pooled and further purified with preparative thin layer chromatography (PTLC) to get compound 1 (30 mg). Similarly, fractions 63-69 (60:40, hexane/ethyl acetate) were also combined and purified using PTLC to afford a mixture (40 mg) of compounds 2 (minor) and 3 (major) and compound 3 (28 mg). Characterization of the isolated compounds was made using spectroscopic techniques.

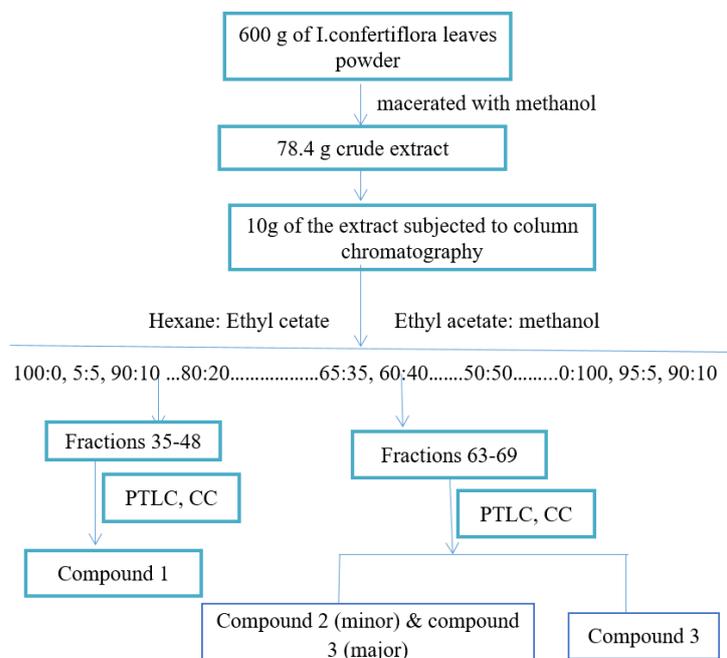


Figure 3. Extraction, isolation, and purification of isolated compounds from the leaves of *I. confertiflora*.

3. Results and Discussion

The ground leaves of *I. confertiflora* was extracted using three solvent systems with different degree of polarity, namely methanol, ethyl acetate, and petroleum ether. The methanol crude extract has a higher percentage yield than ethyl acetate and petroleum ether (Table 2), which was the study's focus.

Table 2. Percentage yield analysis of the extracts.

Solvent system	Plant part taken	Mass of sample taken (g)	Color of the crude extract	Mass of crude (g)	% yield
Methanol	Leaves	100	Dark brown	13	13
Ethyl acetate	Leaves	100	Dark brown	6	6
Petroleum ether	Leaves	100	Dark brown	4	4

3.1. Phytochemical analysis.

The methanol crude extract was selected for the chemical analysis, and the phytochemical constituents detected in the extract were terpenoids, phytosterols, flavonoids, tannin, saponin, and phenols (Table 3) and this data is consistent with previous reports on other species [2,3,4,5]. This extract was subjected to separation and isolation of compounds using chromatographic techniques.

Table 3. Phytochemical profile of the plant leaf methanol extract.

Metabolite	Chemical test	Observed changes	Status (+/-)
Alkaloid	Hagere test	Violate fluid	-
Flavonoid	Lead acetate test	Yellow precipitate	+
Phenol	FeCl ₃ test	Dark brown	+
Tannin	Lead acetate test	Yellow color formed	+
Terpenoids	Salkowisk test	Brown color formed	+
Anthraquinone	HCl test	No precipitate	-
Phlobatannin	HCl test	Yellow PPT	-
Cardiac glycosides	Acidic test	No color change	-
Phytosterols	Liebermann-Burchard's test	Dark brown color formed	+
Saponins	Foam test	Formation of foam	+

(+)-presence and (-) -absence

3.2. Characterization of isolated sesquiterpene lactones.

Frequent chromatographic and chemical investigations of the methanol crude extract led to the isolation of three known sesquiterpene lactones, namely, 4 α , 5 α -epoxyinviscolide (8), graveolide (9), and carabrone (10), and their structure was elucidated using spectroscopic techniques (Supplementary materials). A previous unpublished phytochemical study on the leaf extract reported the isolation and characterizations of three sesquiterpene lactones viz. 9, 10, carpesiolin (11), and two sterols β -sitosterol and stigmasterol [13], respectively (Fig 4).

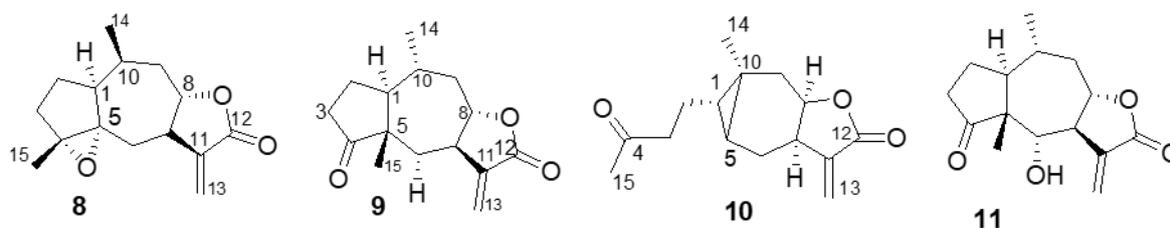


Figure 4. Sesquiterpene lactones isolated from *Inula confertiflora* leaf.

3.2.1. Compound 8 (NP-1).

Compound 8 was isolated as brown solid material (30 mg) (Rf 0.78 in EtOAc:Hex (1:1)) from the leaves extract of *I. confertiflora* at 80:20 fraction of hexane to ethyl acetate on column chromatography. The IR spectrum showed strong absorption bands at 2935 cm^{-1} (-C-H), 1760 cm^{-1} (-C=O) and 1650 cm^{-1} (C=C). ^1H NMR signals at δ 4.01 (1H,m), δ 2.97 (1H,m), 2.53 (1H,m), and 2.08(1H,m) showed methine protons (CH-), of which the first is oxymethine (-OCH). The presence of two CH_3 signals at δ 0.91 (3H,*d*, $J=9.5$ Hz) and 1.33 (3H,*s*) showed their attachment with tertiary and quaternary carbon, respectively. In contrast, two doublet proton signals at δ 5.4 and 6.16 (1H, $J=4$ Hz) indicates the occurrence of one set of exocyclic methylene (CH_2) group. The ^{13}C NMR also supported the existence of a terminal double bond at δ 139.1 and 119.7. Overall spectrum data, including signals of lactone carbonyl and oxygenated carbons at δ 169.9 and 82.5, respectively, established the presence of α -methylene- γ -lactone moiety. Quaternary carbon signals at δ 69.7 and 69.9 suggested the existence of an epoxide ring. Therefore, a comparison of the spectroscopic data with the reported data showed that the compound is a sesquiterpene lactone, 4 α , 5 α -epoxyinuviscolide (8) [13,14] (Table 4).

Table 4. ^{13}C -NMR data of compound 8 and the literature value for 4 α , 5 α -epoxyinuviscolide (CDCl_3 , δ in ppm).

C no	^{13}C -NMR of 10	^{13}C -NMR of 4 α , 5 α -epoxyinuviscolide
1	47.7	47.7
2	30.5	30.6
3	32.7	32.7
4	69.9	69.9
5	69.7	69.7
6	28.9	29.0
7	44.4	44.4
8	82.5	82.6
9	40.4	40.4
10	34.6	34.6
11	139.1	139.1
12	170.0	170.0
13	119.7	118.9
14	14.6	14.7
15	15.5	15.6

3.3. Characterization of compound 9 (NP-2).

Compound 9 was isolated as a yellow-like solid (40 mg) (Rf. 0.56 in EtOAc: Hex (1:1)) from methanol extract of leaves of *I. confertiflora*. The IR spectrum showed absorption bands at 1765 cm^{-1} (γ -lactone), 1718 cm^{-1} (ring ketone), 1653 cm^{-1} (C=C), and 2980/2929 cm^{-1} (C-H), indicating the presence of α , β -unsaturated γ -lactone. It was identified as a minor product in a mixture with carabrone (NP-3), as indicated in NMR spectra. The ^{13}C spectral data at δ 169.7 indicates the presence of lactonic carbonyl group with a bit chemical shift at position C-12, δ 140.3, and δ 80.8 confirms the presence of quaternary carbon (C) with a double bond and methine (CH) directly attached with an oxygen atom, respectively. The signal at position δ 119.7 is assigned to the exocyclic double bond. A comparison of the spectral data with the previous reports indicated the presence of graveolide (9) [13,15] (Table 5).

Table 5. ^{13}C -NMR (CDCl_3 , 400 MHz) data for compound 9 in comparison with the literature value for graveolide.

CN	^{13}C -NMR of 9	^{13}C -NMR of graveolide
1	48.4	48.7
2	24.0	24.1

CN	¹³ C-NMR of 9	¹³ C-NMR of graveolide
3	34.4	34.5
4	-	221.5
5	49.9	50.0
6	35.1	35.2
7	44.6	44.7
8	80.8	80.8
9	43.9	44.1
10	29.4	29.6
11	140.3	140.3
12	169.7	169.8
13	119.7	120.0
14	19.9	20.0
15	21.9	22.0

3.3.1. Compound 10 (NP-3).

Compound 10 was isolated as orange colored solid (28 mg) (Rf. 0.47 in EtOAc: Hex (1:1)) from the methanol extracts of leaves of *I. confertiflora*. The presence of isolated ketone and α , β -unsaturated γ -lactone functionalities were demonstrated from the vibrational frequency of ketone C=O (1703 cm⁻¹), lactone C=O (1750 cm⁻¹), aliphatic C-H (2976/2940 cm⁻¹) and lactone C=C (1640 cm⁻¹). The ¹H NMR spectrum indicated the presence of two singlets at δ 1.03 (H-14) and δ 2.10 (H-15) due to CH₃ groups attached to quaternary carbons (C) besides oxygenated methine proton (OCH) signal at δ 4.73 (1H,m), CH signal at δ 3.12 and exocyclic methylene (CH₂) characteristic doublet proton signals at δ 5.51 ($J=$ 4Hz) and 6.17 ($J=$ 4 Hz)). The triplet signal at δ 2.48 (H-3) showed the presence of a neighboring methylene group (CH₂), and cyclopropane ring protons were also observed at δ 0.42 (1H, m) and 0.33 (1H, m). The ¹³C signals at δ 208.7 and 30.0 (C-15), along with the ¹H signal at δ 2.10 (3H, s, H-15), suggested the presence of an acetyl group that is attached to C-3. Methyl carbon signals at δ 18.1 and 30.0, oxymethine at δ 75.6, cyclopropane ring carbons at δ 17.1 and 22.8, and lactone exocyclic double bond carbon signals at δ 122.4 and 139.0 were observed. The carbon resonances at δ 170.4 and 208.7 are due to a lactone carbonyl and a ketonic carbonyl carbon, respectively. The overall data was in close agreement with that of carabrone (10) [13,16,17] (Table 6).

Table 6. ¹³C-NMR data of compound 10 and the data reported in the literature for carabrone (CDCl₃, δ in ppm).

C no	¹³ C-NMR of 8	¹³ C-NMR of carabrone
1	34.1	34.3
2	23.3	23.3
3	43.4	43.6
4	208.7	208.5
5	22.8	22.90
6	30.6	30.7
7	37.6	37.8
8	75.6	75.6
9	37.2	37.3
10	17.1	17.2
11	139.0	139.0
12	170.4	170.4
13	122.4	122.5
14	18.1	18.2
15	30.0	30.0

4. Conclusions

This study confirms the potential of *I. confertiflora* leaf extract as a source of known bioactive sesquiterpene lactones as that of the other species reported; of which 4 α , 5 α -epoxyinuviscolide, graveolide, and carabrone were isolated and characterized.

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Conflicts of Interest

The authors declare no conflict of interest.

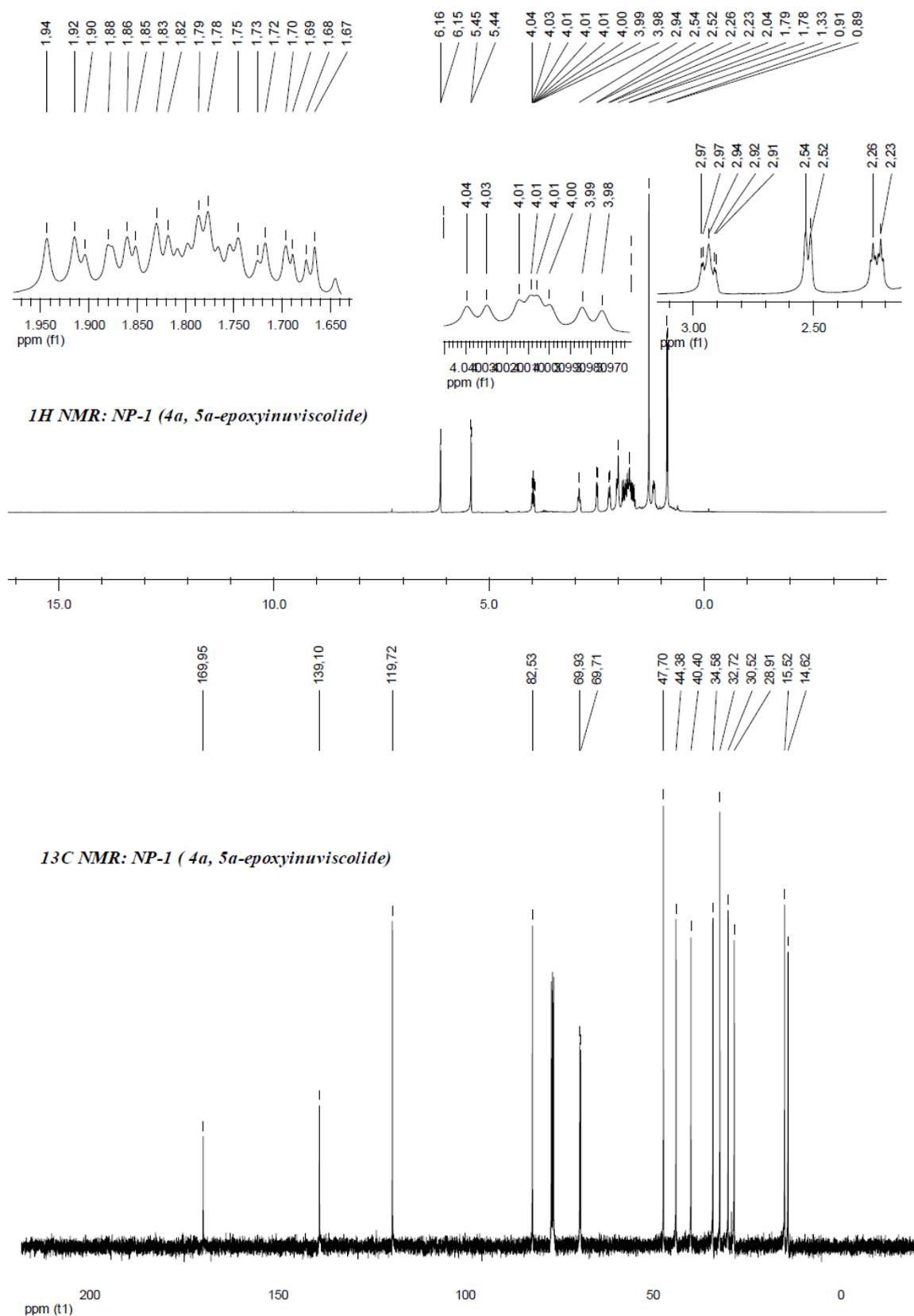
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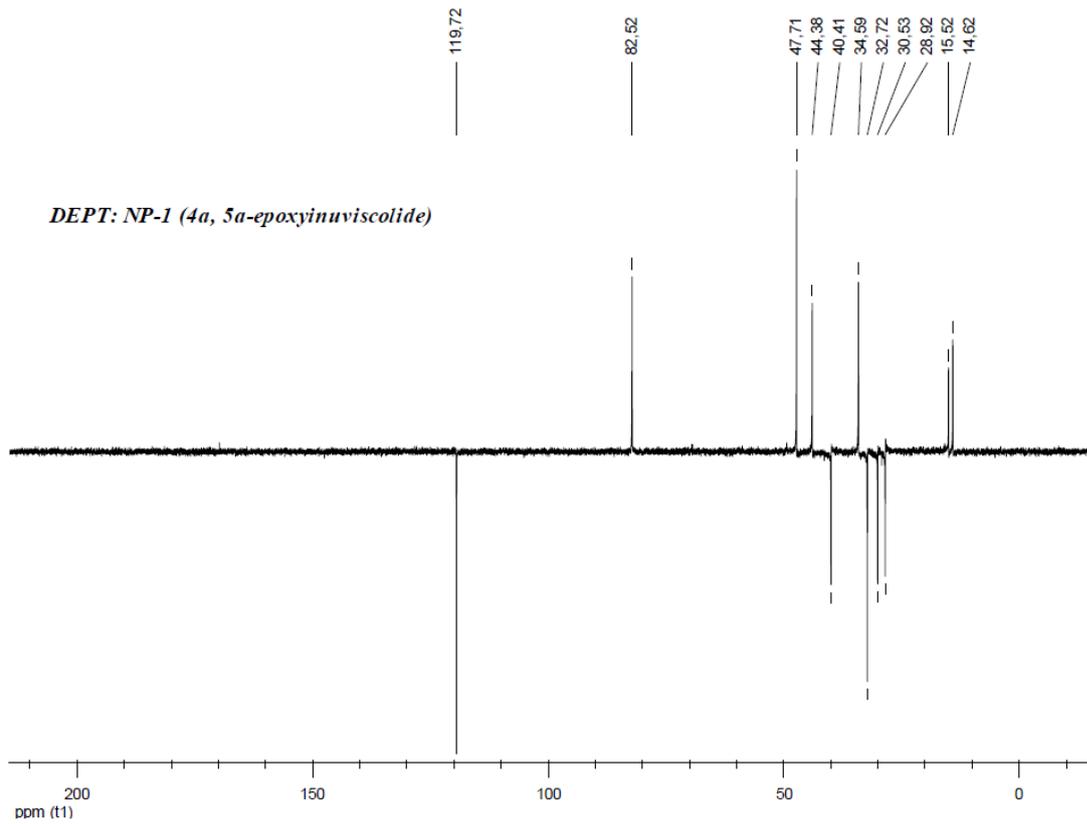
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Supplementary Materials

NP-1: 4a, 5a-epoxyinuviscolide (8)





NP-3: Carabrone (10) and the minor NP-2 (9)

