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Composition of *Xylopia aethiopica* (Dunal) A. Rich essential oils from Cameroon and identification of a minor diterpene: *ent*-13-epi manoyl oxide

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Xylopia aethiopica (Annonaceae) essential oil was extracted from fruits collected in four localities in Cameroon, and analysed by GC/MS and GC/FID. More than sixty compounds were identified with 47.5–84.0% of monoterpenes hydrocarbon, mainly β-pinene and β-phellandrene+1,8-cineole, 6.5–12.9% of oxygenated monoterpenes, 13.8–30.4% of sesquiterpenes, and 0.4–0.6 % of a minor unidentified diterpene. Trials of purification by column chromatography, followed by GC/MS and NMR analysis led to the identification of ent-13-epi manoyl oxide which is reported for the first time as a minor component in *X. aethiopica* essential oil.
Keywords. *Xylopia aethiopica*, essential oil, β-pinene, β-phellandrene+1,8-cineole, *ent*-13-epi manoyl oxide.

Composition des huiles essentielles de *Xylopia aethiopica* (Dunal) A. Rich du Cameroun et identification d'un diterpène : ent-13-epi manoyl oxyde. L'huile essentielle des infrutescences *de Xylopia aethiopica* (Annonacée), de quatre localités du Cameroun, a été analysée par GC/MS et GC/FID. Plus de soixante composés ont été identifiés avec 47,5–84,0 % de monoterpènes hydrocarbonés, principalement le β-pinène et un mélange de β-phellandrène et de 1,8-cinéole, 6,5–12,9 % de monoterpènes oxygénés, 13,8–30,4 % de sesquiterpènes, et 0,4–0,6 % d'un diterpène non identifié. Les essais de purification sur colonne chromatographique et des analyses par GC/MS et RMN ont permis d'identifier ce diterpène comme étant le ent-13-epi manoyl oxyde qui est rapporté pour la première fois dans l'huile essentielle des fruits de *X. aethiopica*.
Mots-clés. *Xylopia aethiopica*, huile essentielle, β-pinène, β-phellandrène+1,8-cinéole, *ent*-13-épi manoyl oxyde.

1. INTRODUCTION

Xylopia aethiopica is a tree of more than 20 m of height and 60–75 cm of diameter which grows in the forest zone and especially along the rivers in arid areas. The fruit is a slightly hooked cylindrical pod reaching 2–3 mm in width. The mature fruits of green colour take a brown-black coloration after drying and are used as spices (often instead of pepper), in traditional medicine (against the flu, the bronchitis and the dysentery).

Several studies are reported on biological activity of *X. aethiopica*. Fruit powder, its essential oil (Okonkwo, Okoye, 1996; Ngamo *et al*., 2001; Kouninki *et al*., 2005) or leave essential oil (Asawalam *et al*., 2006) can be used against cowpea bruchid *Callosobruchus maculatus* (Fab.) (*Coleoptera: bruchidae*) or maize weevil *Sithophylus zeamais* Motsch. (*Coleoptera: curculionidae*). *X. aethiopica* is also active against the termites and other bugs who tackle wood (Ladjide *et al*., 1995). The microbiological activity of *X. aethiopica* essential oil against *Escherichia coli*, *Staphylococcus aureus* or *Aspergillus flavus*, among other microorganisms, has been well established (Tatsadjieu *et al*., 2003; Asekun, Adeniyi 2004; Konnings *et al*., 2004).

Among the compounds that confer to *X. aethiopica* its biologic properties one can mention, the diterpenes belonging to the kauranes, the trachylobanes and the kolovanes families (Hasan *et al*., 1982; Harrigan *et al*.,

1994). It is also noticed that the features of the ether extract of *X. aethiopica* are favorable to its incorporation in the resins used for the manufacture of the paintings (Ajiwe *et al.*, 1998).

The composition of fruit essential oil of *X. aethiopica* given in the literature, shows that it is constituted of monoterpenes hydrocarbon. These compounds are represented mainly by β-pinene 37.0–40.5% (Tomi *et al.*, 1996), 12.0–42.0% (Ayedoun *et al.*, 1996), 18.3% (Jirovetz *et al.*, 1997) 9.9–19.1% (Keita *et al.*, 2003) or by sabinene 36.0% according to Poitou *et al.* (1996). Germacrene D is the most important sesquiterpene and the oxygenated compounds are mainly the 1,8-cineole and the terpinen-4-ol. A survey undertaken on *X. aethiopica* essential oil from Egypt showed very particular composition with more than two third of oxygenated compounds 23.4% of terpinen-4-ol, 16.3% of 1,8-cineole and 11.1% of α-terpineol (Karawya *et al.*, 1979). A similar composition with oxygenated monoterpenes (15.1% of 1,8-cineole, 6.6% of terpinen-4-ol) has been reported in essential oil from Nigeria (Asekun, Adeniyi, 2004).

The aim of the present work is to study the composition of *X. aethiopica* essential oil from four localities in Cameroon, and to emphasize the occurrence of a diterpene never mentioned in *X. aethiopica* essential oil which appears to be *ent*-13-epi manoyl oxide.

2. EXPERIMENTAL

2.1. Essential oil extraction

Dried fruits of *X. aethiopica* were obtained from four localities: Bafoussam, Douala, Ngaoundere and Yaounde.

They were ground and subjected to hydrodistillation during four hours using a Clevenger-type (**Figure 1**) apparatus. Yellowish essential oils were then obtained.

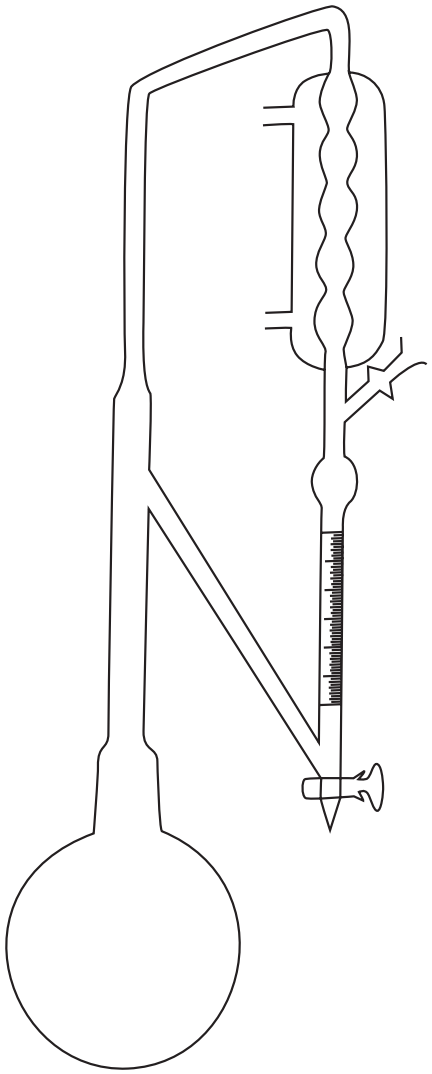

Figure 1. Clevenger-type apparatus — *Appareil de type Clevenger.*

2.2. Extraction and purification of a natural diterpene

The isolation of enriched fractions containing a natural diterpene were carried out on n-hexane extracts from ground fruits since its content in the essential oil is too low (<1%). Ground fruits (30 g) were mixed with 200 ml of n-hexane and the raw extract (300 mg) was fractionated by successive column chromatography purifications with silica gel G60 (5% water content). Several preliminary trials have been undertaken and the optimized protocol is summarized in **figure 2**.

2.3. Gas chromatography and gc-ms analyses

GC analyses were performed on an Agilent 6890 series apparatus fitted with a split/splitless injector (splitless mode). The operating conditions were as follows: 30 m*0.25 mm HP 5MS (crosslinked 5% phenyl dimethylsiloxane), film thickness: 0.25 μm, temperature programme: from 40°C–230°C at 5°C/min with a final hold of 5 min. at 280°C. Helium at 49.9 KPa was used as carrier gas and the FID detector was maintained at 250°C.

The oil constituents were identified on the basis of their retention and fragmentation data by using GC/MS analytical conditions similar to that of GC-FID. The mass spectra were recorded on a Agilent 5973 mass spectrometer coupled to an Agilent gas chromatograph (EI mode 70eV, source temperature 230°C, scanned mass ranged 35 to 350 amu). The characteristic fragmentation patterns have been analysed and compared to those of Wiley 275.L database. The retention data (retention indices) were compared to those of Adams (2001) and Joulain and König (1998).

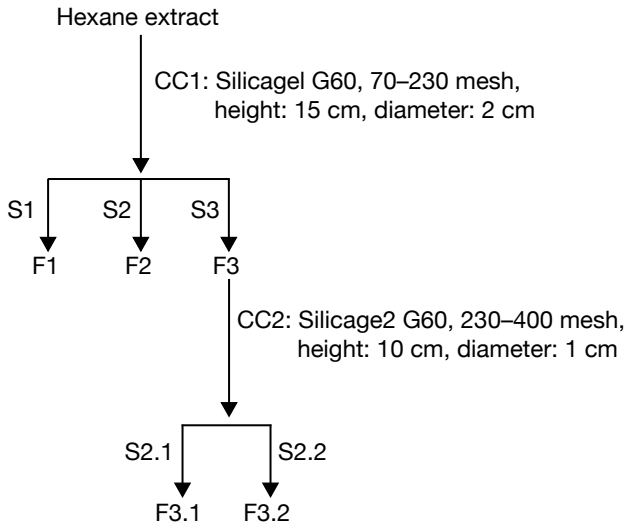

Figure 2. Column chromatography of hexane extract of *Xylopia aethiopica — Chromatographie sur colonne de l'extrait à l'hexane de* Xylopia aethiopica.

CC: Column Chromatography — *Colonne chromatographique*; S1: 20 ml of hexane — *20 ml hexane*; S2: 30 ml of hexane/diethyl ether: 6/4 — *30 ml hexane/ether éthylique: 6/4*; S3: 20 ml of hexane/diethyl ether: 1/1 — *20 ml hexane/ether éthylique: 1/1*; S2.1: 7.5 ml hexane/diethyl ether: 6/4 — *7.5 ml hexane/ether éthylique: 6/4*; S2.2: 2.5 ml hexane/diethyl ether: 6/4 — *2.5 ml hexane/ether éthylique: 6/4*; F1, F2, F3, F3.1, F3.2: collected fractions — *fractions collectées*.

2.4. NMR analysis

Fraction F3.2, collected during column chromatography, was analysed by NMR (CDCl3, 500MHz) in the Unit of Structural Chemistry and Reaction Mechanisms (CSTR) of the Catholic University of Louvain-la-Neuve (Belgium).

3. RESULTS AND DISCUSSION

The four analyzed samples contained mainly monoterpenes hydrocarbons (41.76–77.04%), in particular α-pinene (3.38–13.65%), β-pinene (8.22–44.11%). The β-pinene appears like an important compound in the essential oil of *X. aethiopica* since it is also the major compound in the oil of Guinea with 37.00 to 40.50% (Tomi *et al.*, 1996), of Mali with 9.90% (Keita *et al.*, 2003) and of Cameroon with 18.30% (Jirovetz *et al.*, 1997). However the sample from Bafoussam is particular with 31.42% of β-phellandrene+1,8-cineole against 8.77 to 17.20% for the other samples. With the forementioned analytical conditions, β-phellandrene and 1,8-cineole coeluted, they were therefore summed in **table 1**. It is noteworthy that the essential oil originating from Bafoussam contained 14.56% of unidentified compounds each representing less than 1%. The origin of the fruits could explain the differences observed, but also the treatments that the fruits undergo after the harvest. Indeed, according to Ayedoum *et al.* (1996), the α-pinene and the sabinene can vary respectively from 4 to 16% and 3 to 35% according to whether the fruits are boiled or smoked before the drying.

One particular compound appeared in small amount (less than 1%) at a retention time of 34.2 min. Its molecular ion suggests a diterpene. The molecule, of moderate volatility, was systematically observed after long distillation times and only Jirovetz *et al.* (1997) mentioned the occurrence of such a compound in the essential oil of *X. aethiopica* without having proposed any identification. Successive column chromatography purifications (**Figure 2**) led to the recovery of 58.50 mg of an enriched fraction called F3.2 (**Figure 3**) which contained exclusively diterpenes, notably a derivative of manoyl oxide type. In agreement with Angelopoulou *et al.* (2001), the recorded mass spectra and the m/z (mass to charge ratio)=275 and 257 intensity ratio (ion m/z=257 higher than ion m/z=275), oriented identification toward the ent-13-epi manoyl oxide (**Figure 4**) rather than manoyl oxide where the two ions are almost equal. Comparing to the 1H NMR spectrum of pure *ent*-13-epi manoyl oxide (Demetzos *et al.*, 2002), the recorded 1H NMR spectrum (**Figure 5**) showed the occurrence of the doublets H-15 cis and H-15 trans. A selective irradiation at 6 ppm confirmed the presence of H-14 since the doublets H-15 were reduced to two singlets by suppression of H-14/H-15 coupling. This confirmed well that the molecule of interest is the ent-13-epi manoyl oxide. Successive injections of the essential oil and the purified product revealed the same kovats index (KI=1992).

4. CONCLUSION

More than 60 compounds were identified in the four samples of *X. aethiopica* essential oils which show the complexity of this natural extract with insecticide activity (Ngamo *et al.*, 2001; Kouninki *et al.*, 2005). The main chemical compounds are: β-pinene, β-phellandrene+1,8-cineole, α-pinene, terpinen-4-ol and germacrene D with different content in each sample. With GC-MS and NMR investigations, it was possible to unambiguously identify the *ent*-13-epi manoyl oxide, a diterpene which is reported for the first time in *X. aethiopica* essential oils. Nevertheless due to its very low proportion in all analysed hydrodistillates it is not established that this molecule could play a significant role in the essential oil activity.

Tableau 1. Composition (%) de l'huile essentielle de *Xylopia aethiopica* provenant de quatre localités du Cameroun.

	Compounds	**KI**	**Baffoussam**	**Douala**	**Ngaoundere**	**Yaounde**
	Essential oil yield % (v/w)		**1**	**8**	**5**	**7**
1	α-thujene	914	0.21	0.20	1.48	0.55
2	α -pinene	920	3.38	13.65	10.20	12.43
3	α -fenchene	934	0.65	-	-	-
4	β-pinene	963	8.22	39.39	38.17	44.11
5	α-phellandrene	979	1.41	0.68	0.50	0.75
6	α-terpinene	1005	0.30	0.34	1.57	1.00
7	p-cymene	1013	tr	tr	1.06	0.41
8	β-phellandrene + 1,8-cineole	1019	31.42	17.20	8.77	13.89
9	Z-β-ocimene	1028	-	0.72	1.13	1.12
10	cis-β-ocimene	1047	0.56	0.69	2.81	1.76
11	γ-terpinene	1055	0.15	0.17	0.92	0.43
12	terpinolene	1077	0.16	0.22	0.79	0.45
13	allo-ocimene	1088	-	0.17	0.67	0.14
14	trans-sabinene hydrate	1089	0.40	0.40	0.64	0.30
15	cis-p-menth-2-en-1-ol	1109	0.28	0.13	0.31	-
16	trans-pinocarveol	1125	0.56	0.89	1.48	0.41
17	myroxyde E	1130	0.32	0.22	0.62	0.62
18	isopulegol	1141	0.21	0.28	0.46	0.19
19	β-pinene oxide	1145	0.12	0.09	0.17	-
20	p-mentha-1,5-dien-8-ol	1152	0.12	0.09	0.17	0.20
21	terpinene-4-ol	1159	1.54	1.92	4.55	2.73
22	cryptone	1166	1.01	0.45	0.35	0.24
23	α-terpineol	1171	0.76	0.64	0.91	0.83
24	myrtenal	1175	0.49	0.79	1.69	0.75
25	verbenone	1186	0.19	0.10	0.61	-
26	trans carveol	1196	0.13	0.07	-	0.16
27	cuminal	1216	0.62	0.10	0.22	-
28	piperitone	1232	0.13	-	0.15	0.09
29	bornyl acetate	1269	0.47	0.07	0.15	-
30	thymol	1277	0.26	0.09	0.21	-
31	p-cymen-7-ol	1290	0.33	-	-	-
32	2E,4Z-decadienal	1302	0.68	0.23	0.19	-
33	δ-elemene	1325	1.95	1.88	1.59	0.76
34	α-cubebene	1337	0.90	0.19	0.38	0.39
35	longycyclene	1358	0.26	0.12	0.21	0.11
36	α-copaene	1363	3.70	0.67	0.58	2.41
37	β-bourbonene	1372	0.14	-	-	-
38	β-cubebene	1377	0.30	-	-	-
39	cyperene	1386	0.39	0.11	-	0.12
40	Z-caryophyllene	1405	1.68	1.32	1.05	0.95
41	β-duprezianene	1420	1.11	0.61	0.68	0.31
42	α-guaiene	1425	0.11	-	-	-
43	cis-prenyl limonene	1430	0.41	0.34	0.31	0.24
44	dehydro aromadendrene	1464	1.86	0.55	0.50	0.22
45	germacrene D	1468	5.34	5.07	3.61	3.69
46	β-selinene	1473	0.48	0.12	0.09	0.10
47	10-epi-zonarene	1481	1.28	0.32	0.33	0.12
48	cis-cadina-1,4-diene	1486	0.98	0.50	0.68	0.38
49	α-muurolene	1493	0.23	0.11	0.15	0.34
50	Z-γ-bisabolene	1500	1.47	0.33	0.37	-
51	δ-cadinene	1509	2.62	0.64	0.80	0.61
52	E-γ-bisabolene	1518	0.23	0.09	0.13	0.13
53	α-cadinene	1523	0.37	-	0.11	-
54	α-calacorene	1528	0.21	-	-	-
55	selina-3,7(11)-diene	1535	0.22	0.29	0.18	-
56	methyl perillate	1379	0.93	0.53	0.48	0.55
57	cabreuva oxyde A	1435	0.35	0.21	0.26	0.24
58	spathulenol	1563	0.29	0.13	0.35	0.10
59	caryophyllene oxyde	1568	0.42	0.11	0.17	0.08
60	thujopsan-2-α-ol	1574	0.33	0.19	0.13	0.08
61	geranyl-2-methyl butanoate	1578	0.44	0.46	0.20	0.09
62	epoxy-allo alloaromadendrene	1613	1.36	0.84	0.43	0.44
63	***ent*-13-epi manoyl oxide**	**1992**	**tr**	**0.41**	**0.60**	**0.40**

KI: Kovats Indice; tr: trace (<0.10%) ; V/W: Volume/Weight

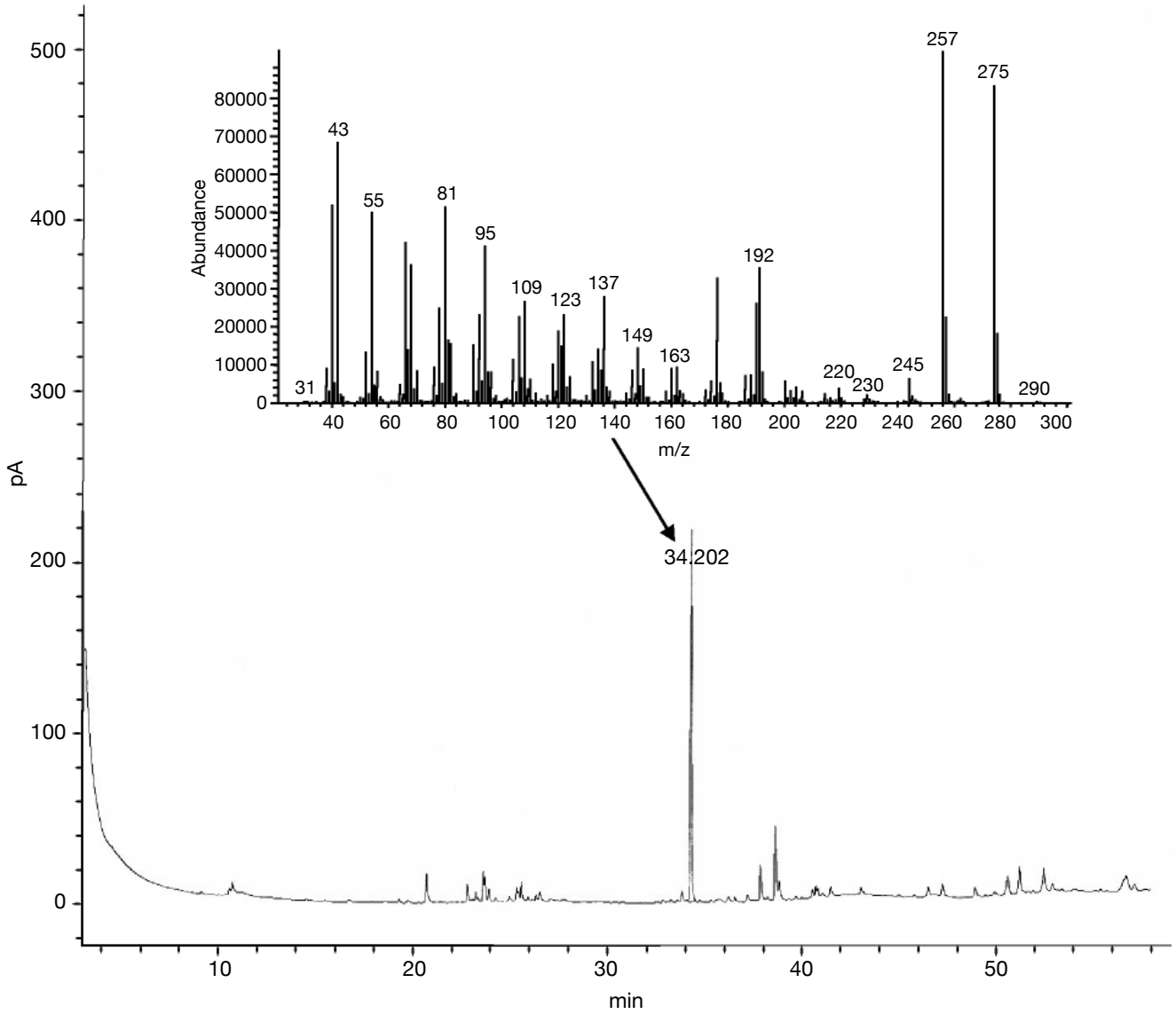

Figure 3. Chromatograph of fraction F3.2 and mass spectrum of *ent*-13-epi manoyl oxide — *Profil chromatographique de la fraction F3.2 et spectre de masse du* ent-*13-epi manoyl oxyde*.

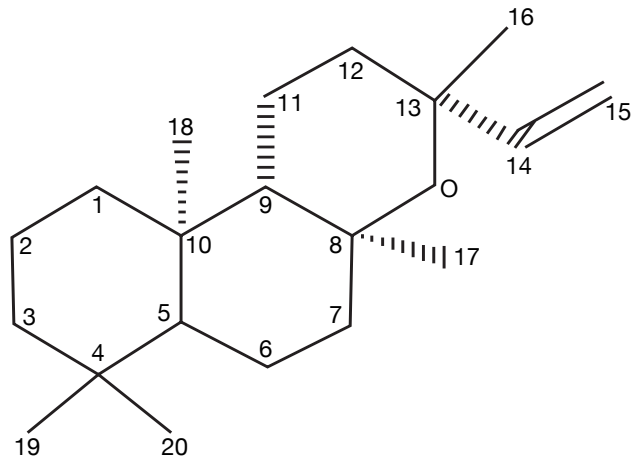

Figure 4. Formule of the *ent*-13-epi manoyl oxide — *Formule du* ent-*13-epi manoyl oxyde*.

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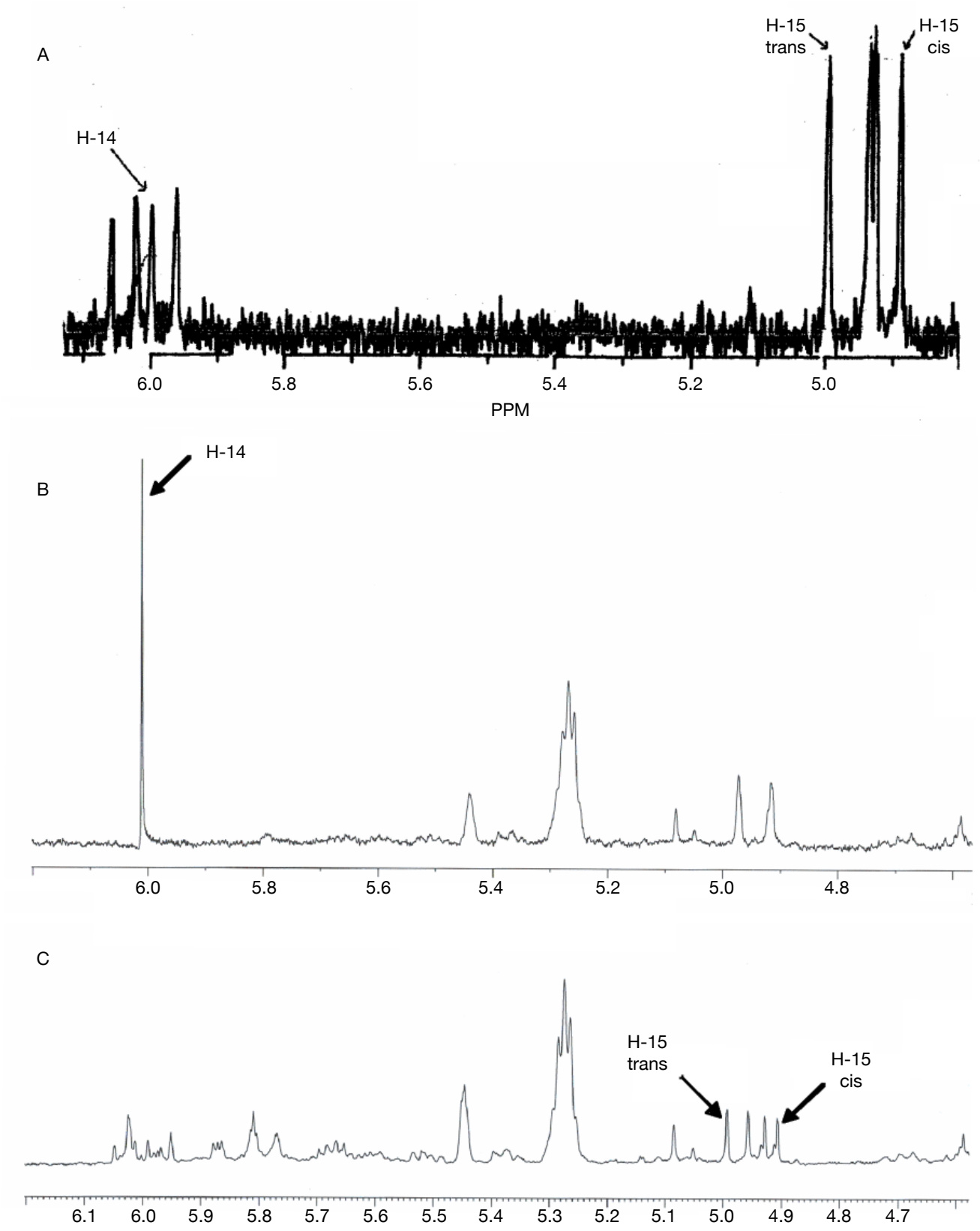

Figure 5. [1]H NMR spectra of pure *ent*-13-epi manoyl oxide (A) and of fraction F3.2 (B,C) — *Spectres RMN du* ent-*13-epi manoyl oxide et de la fraction F3.2* (Demetzos *et al.*, 2002).

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