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VOLATILE CONSTITUENTS OF *KYLLINGA ERECTA* S

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ABSTRACT. The volatile constituents of the rhizomes and the aerial parts of *Kyllinga erecta* S, a medicinal plant, were analysed by GC and GC-MS, and by spectroscopic techniques. The dichloromethane extract of the rhizomes was found to contain a large amount of diterpenoids from labdanic series, such as manoyloxide, and oxo- and hydroxymanoyloxide derivatives. The extract of the aerial parts was characterised as the sesquiterpenoids compounds; the major constituent was identified as cyperene.

KEY WORDS: *Kyllinga erecta* S, Volatile constituents of *Kyllinga erecta* S, Sesquiterpenoids, Cyperene, Manoyloxide, Oxomanoyloxide derivatives, Hydroxymanoyloxide derivatives

INTRODUCTION

Kyllinga erecta (Cyperaceae family) is an annual herb, which is widespread throughout tropical and subtropical zones. In Chad, this plant is found in the south country swamps and on the riversides [1, 2]. The rhizomes of this herb which exhale a strong and pleasant odour, are sold in town markets (more than 10 tons are sold every year), for domestic use and cosmetic purposes (1 kg/7 US $). In African traditional medicine, the volatile oil of the plant is used in the treatment of itching and hepatitis, and the aqueous extract has been shown to possess tonic, antifebrile and antidiabetic properties, and may be used as antidote to poison [3]. In continuation of our studies on the chemical composition of the essential oil of *K. erecta* S [4-8], we have now investigated the dichloromethane extracts of the aerial part and the rhizomes.

EXPERIMENTAL

Plant material. Plants of *K. erecta* growing wild in Moundou (Southern Chad) were collected and authenticated. A voucher specimen is deposited at the herbarium of "Laboratoire Vétérinaire et Zootechnique de Farcha" in N'Djamena.

Extraction. From the samples of air-dried plant material, rhizomes and aerial parts were picked up. 50 g of the crushed rhizomes and 500 g of the aerial parts of the plant sample were each subjected to dichloromethane extraction for 5 h using a Soxhlet apparatus.

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Analysis methods of the extracts

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The extracts of the rhizomes and the aerial parts of *K. erecta* were analysed by GC using a Girdel Model 30 instrument equipped with FID, and by combined GC-MS using a Hewlett Packard system. The GC column used was a fused silica capillary column with DB-1 bonded phase. The detector of the mass spectrometer is a quadrupole system (electron energy: 70 eV). The operating conditions were as described elsewhere [4].

Column chromatography. The dichloromethane extract of the rhizomes (8.4 g) was subjected to column chromatography over silica gel using hexane-diethyl ether in gradient elution. Some fractions were joined together following the TLC analysis. Eight fractions were obtained.

High performance liquid chromatography. The fractions collected from the dichloromethane extract were subjected to preparative HPLC using Waters Model 510, differential refractometer Waters 410 with column Bondapack C18 (19 x 150 mm). The eluting solvent was MeOH-H_2O (4:1).

Nuclear magnetic resonance spectrometry. ^1H and ^{13}C NMR spectra of the purified fractions were recorded using Bruker AM-200 or AM-300 spectrometers. All spectra were obtained in deuterochloroform as solvent. Assignments of the ^{13}C NMR chemical shifts were made with the aid of broad-band proton decoupling and experiments of distortionless enhancement by polarisation transfer (DEPT) using a "flip angle" of 135°.

RESULTS AND DISCUSSION

Extraction with dichloromethane of the rhizomes and the aerial parts of *K. erecta* yielded 4.8% of a syrupy crude mixture and 1.8% of an amorphous residue, respectively.

Careful analysis by GC-MS of the extracts and by NMR spectra of the components isolated from the rhizome extract allowed us to identify the components from *K. erecta* (Table 1).

All the compounds were located by GC-MS. Analysis of the mass spectra showed the presence of epimeric pairs (**1** and **2**, **3** and **4**, **6** and **9**, and **8** and **10**) for which the mass spectra were similar (Table 2). The mass spectra of the two epimers **1** and **2** matched the spectra for manoyloxide and epi-13-manoyloxide reported in MS library [14]. The fragmentation of all compounds **1-10** agrees with the fragmentation proposed by Enzell and Ryhage [14] for manoyloxide derivatives (Figure 1). The peak associated with k ion is prominent for the compounds of these labdanic diterpenoid series with a C-13 vinyl group. The peak corresponding to e ion is often significant in the mass spectra of alcohols **6** and **9**, **8** and **10**, and **7** that have no oxygen substituents in the A ring.

The ^1H and ^{13}C-NMR characteristics and the chemical correlations established the structure of these labdanic diterpenoids. Oxidation by Jone's reagent of alcohols **6**, **9** and **8** gave three ketones **3**, **4** and **5**, respectively. Ketones **3** [5, 12, 13] and **5** [5] are identified by their spectral characteristics (Tables 2-4).

The ^1H NMR spectra of compounds (**1-3**, **5-9**) showed signals for five tertiary methyl groups and an ABX system corresponding to a vinyl group attached to a quaternary sp^3 (Figure 1). The ^{13}C NMR spectra of compounds (**1-3**, **5-10**) confirm the precedent analysis. The elucidation of the structures of **1** and **2** is confirmed by comparison of their ^{13}C NMR data with those described

in the literature [15]. The strong differences observed in the chemical shifts at C-8, C-9, C-16 and C-17 of **1** and **2** allowed to easily distinguish manoyloxide and epi-13 manoyloxide derivatives.

Table 1. Quantitative composition of the extracts of *K. erecta*.

Component	Rhizomes		Aerial parts	Methods of identification[a]
	Oil [4, 5]	Extract	Extract	
Monoterpene hydrocarbons	0.2	t	0.8	
β-Pinene	0.2	t	-	RI, MS
p-Cymene	-	-	0.8	RI, MS
Oxygenated monoterpenes	0.5	t	-	
1,8-Cineole	0.2	t	-	RI, MS
Methyl-thymol	0.3	t	-	RI, MS
Sesquiterpene hydrocarbons	11.7	5.1	39.9	
α-Copaene	0.2	t	1.5	RI, MS
β-Bourbonene	-	-	1.1	RI, MS
β-Elemene	-	-	1.1	RI, MS
Cyperene	9.4	4.9	29.0	MS, ^{1}H, ^{13}C [9]
β-Caryophyllene	-	-	2.3	RI, MS
Sativene	1.4	-	-	RI, MS
β-Selinene	0.7	-	-	RI, MS
α-Humulene	-	0.2	-	RI, MS
Germacrene-D	-	t	4.9	RI, MS
Oxygenated sesquiterpenes	11.0	3.6	15.8	
Cariophyllene oxide	-	0.9	1.9	RI, MS
Humulene oxide	-	0.2	-	RI, MS
Patchoulenone	-	0.2	-	RI, MS
Spatulenol	-	0.2	-	RI, MS
Cyperotundone (= cyperenone) [10, 11]	10.2	1.8	3.4	MS, ^{1}H, ^{13}C
Hexahydrofarnesylacetone	-	0.5	10.5	RI, MS
Labdanic diterpenoids	53.3	85	9.4	
Manoyloxide (**1**)	48	53.2	5.7	MS, ^{1}H, ^{13}C [4]
13-Epimanoyloxide (**2**)	4.3	3.7	1.8	MS, ^{1}H, ^{13}C [4]
11-Oxo-manoyloxide (**3**)	3.5	6.5	0.5	MS, ^{1}H, ^{13}C [5, 12, 13]
11-Oxo-13-epimanoyloxide (**4**)	-	1.2	0.2	MS[b]
1-Oxo-manoyloxide (**5**)	-	0.5	0.4	MS, ^{1}H, ^{13}C [5]
11α-Hydroxymanoyloxide (**6**)	7.5	8.9	0.4	MS, ^{1}H, ^{13}C [5]
16-Hydroxymanoyloxide (**7**)	-	2.1	-	MS, ^{1}H, ^{13}C [7]
1β-Hydroxymanoyloxide (**8**)	-	4.6	0.2	MS, ^{1}H, ^{13}C [5]
11α-Hydroxy-13-epimanoyloxide (**9**)	-	1.9	-	MS, ^{1}H, ^{13}C [8]
1β-Hydroxy-13-epimanoyloxide (**10**)	-	3.4	0.2	MS, ^{13}C[c]
Acids	3.7	2.5	12.6	
Hexanoic	0.3	-	1.2	RI, MS
Decanoic	-	0.2	-	RI, MS
Octanoic	-	-	1.5	RI, MS
Dodecanoic	1.2	-	0.4	RI, MS
Tetradecanoic	0.5	0.2	1.6	RI, MS
Pentadecanoic	-	0.8	2.5	RI, MS
Hexadecanoic	1.7	1.3	5.4	RI, MS
9,12-Octadecadienoic	-	0.4	0.7	RI, MS

[a]RI: Retention Index in DB-1 compared with authentic compounds and with literature data, MS: mass spectra, ^{1}H: ^{1}H NMR, ^{13}C: ^{13}C NMR. [b] Mass spectra compared with authentic ketone obtained from alcohol (**9**) by oxidation with Jone's reagent. [c]^{13}C NMR from a mixture of alcohols **10** and **8**.

Table 2. Mass spectral data of manoyloxide and 13-epimanoyloxide derivatives.

Compound	1, 2	3, 4	5	6, 9	7	8 10
M	absent	304[1]	absent	306[1]	absent	306[1]
a[2]	275 (100)		289 (76)	291	275 (19)	291
M-(15+18)	257		271 (32)	273	257 (100)	
M-(18+27)	245		259 (1)			
M-h[2]	220	234[1]	234 (2)			
g[2]	205				205 (3)	
k[2]	192	192[1]	206 (28)	192[1]		208
k-15	177	177	191 (8)	177		193
e[2]	137	137	137 (8)	137	137 (39)	
Others peaks		236, 221, 219, 208, 109 (100)	70 (10), 43 (100)	255, 203, 43 (100)	291 (1), 258 (26), 123 (20), 105 (13)	201, 43 (100)

[1]The intensity of this peak is generally low. [2]See fragmentation in Figure 1.

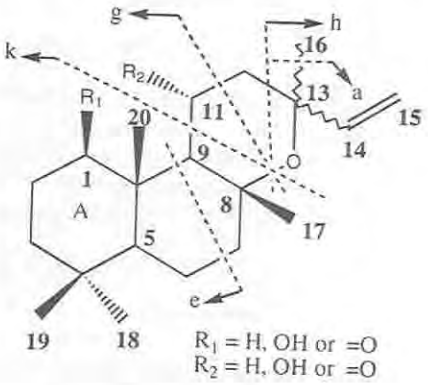

Figure 1. Fragmentation of manoyloxide and 13-epimanoyloxide derivatives.

The configurations of alcohols **6-10** were established by comparison of their spectroscopic properties with those of known compounds.

The ^{1}H NMR spectrum of **6** was very different from that of its known 11-β-hydroxymanoyloxide epimer [16] where the protons of Me-16 (δ 1.35), Me-17 (δ 1.53) and Me-20 (δ 1.11), are deshielded by the 1-3 diaxial interaction with the axial hydroxyl group. Therefore, in accordance with the fact that an axial proton in a tetrahydropyran ring is more shielded than its equatorial counterpart, the H-11β signal (δ 3.94) of **6** resonated at a higher field than that of the H-11α signal (δ 4.38) of the 11-β hydroxymanoyloxide.

The ^{1}H NMR data of **9** showed that the compound contained a secondary hydroxyl group (δ 4.08 ddd). The multiplicity of the methine proton could be rationalised only if the hydroxyl group is located at C-6 or C-11. Evidence for the location of the alcohol group at C-11 came from a comparison of the ^{13}C-NMR spectra of **9** and **2**. The lowfield shift of C-11 in **9** (δ 65.5) compared to that of **2** (δ 15.9) was attributed to the deshielding effect of a hydroxyl group. The main differences between the ^{13}C-NMR spectra of **9** and its 13-epimer **6** were observed for the resonances corresponding to carbons at C-16, C-9 and C-8. The α (equatorial) configuration of the secondary hydroxyl at C-11 was indicated from the downfield chemical shift of H-11 (δ

4.08), which was comparable to those observed for other 13-epi-manoyloxide derivatives containing an 11α-hydroxyl group [8].

Table 3. ^{13}C NMR data (δ ppm) of manoyloxide and 13-epimanoyloxide derivatives

Compound	1	2	3	5	6	9	7	8	10
C-1	39.0	39.4	41.9	216.4	40.0	41.2	38.9	79.8	77.2
C-2	18.6	18.7	18.4	36.3	18.4	18.7	18.5	29.6	29.9
C-3	42.1	42.2	43.3	42.1	41.9	41.9	42.0	40.0	
C-4	33.2	33.3	33.4	33.2	33.3	33.5	33.2	33.0	
C-5	56.4	56.4	55.8	56.8	56.4	56.3	56.4	55.0	
C-6	20.0	20.0	19.7	20.54	20.0	19.9	20.2	19.9	20.0
C-7	43.3	43.1	39.5	42.3	43.6	43.6	43.6	43.2	43.3
C-8	75.1	76.0	77.3	75.0	74.5	76.2	76.3	75.1	76.1
C-9	55.6	58.5	66.8	49.0	62.3	63.3	52.8	55.0	58.9
C-10	37.0	37.0	37.2	51.6	37.9	38.6	37.2	42.8	
C-11	15.4	15.9	207.7	17.5	65.0	65.5	14.5	18.5	18.9
C-12	35.7	34.8	50.2	35.7	43.0	43.8	27.2	35.0	
C-13	73.2	73.2	75.0	73.7	73.1	73.6	75.6	73.30	76.0
C-14	148.0	147.7	146.7	147.9	148.3	148.8	144.0	147.9	147.5
C-15	110.3	109.5	112.2	111.3	112.3	110.0	114.0	110.4	109.6
C-16	28.5	32.7	31.3	28.1	33.8	31.5	68.5	29.1	32.6
C-17	25.5	23.9	28.0	25.3	27.8	26.2	25.8	25.8	24.2
C-18	33.4	33.4	33.5	31.8	33.4	33.6	33.4	32.9	33.4
C-19	21.4	21.3	21.6	22.7	21.5	21.5	21.5	21.0	20.8
C-20	15.3	15.9	15.5	15.0	16.2	16.4	15.1	11.3	11.8

Table 4. Pertinent ^{1}H NMR data (δ ppm, coupling constants Hz) of manoyloxide and 13-epimanoyloxide derivatives.

Compound	1	2	3	5	6	9	7	8
Me-18-20	0.78 (s)	0.72 (s)	0.81 (s)	0.94 (s)	0.81 (s)	0.74 (s)	0.80 (s)	0.79 (s)
	0.79 (s)	0.78 (s)	0.87 (s)	1.04 (s)	0.87 (s)	0.81 (s)	0.80 (s)	0.85 (s)
	0.85 (s)	0.85 (s)	1.03 (s)	1.18 (s)	0.88 (s)	0.85 (s)	0.86 (s)	0.86 (s)
Me-16	1.27 (s)	1.13 (s)	1.29 (s)	1.30 (s)	1.22 (s)	1.19 (s)	-	1.25 (s)
Me-17	1.29 (d, 0.9)	1.22 (s)	1.32 (s)	1.34 (s)	1.29 (s)	1.23 (s)	1.28 (s)	1.30 (s)
Vinyl group protons	4.92 (10.7, 1.6)	4.91 (11.1, 0.9)	5.06 (10.7, 1.1)	4.86 (10.8, 1.4)	5.07 (10.5, 2.0)	4.86 (10.8, 0.9)	5.12 (10.7, 1.5)	4.93 (10.7, 1.6)
	5.17 (17.4, 1.6)	5.00 (18.1, 0.9)	5.24 (17.3, 1.1)	5.07 (17.3, 1.4)	5.43 (17.1, 2.0)	5.03 (17.6, 0.9)	5.26 (17.4, 1.5)	5.16 (17.4, 1.6)
	5.87 (17.4, 10.7)	6.01 (18.1, 11.1)	5.95 (17.3, 10.7)	5.81 (17.3, 10.8)	6.04 (17.1, 10.5)	5.89 (17.6, 10.8)	5.81 (17.4, 10.7)	5.89 (17.4, 10.7)
Carbinolic proton					3.94 (ΣJ = 13.4)	4.08 (ddd, 8.7, 5.8, 5.3)	3.34 (d, 11.1) 3.28 (d, 11.1)	3.37 (t, 7.8)

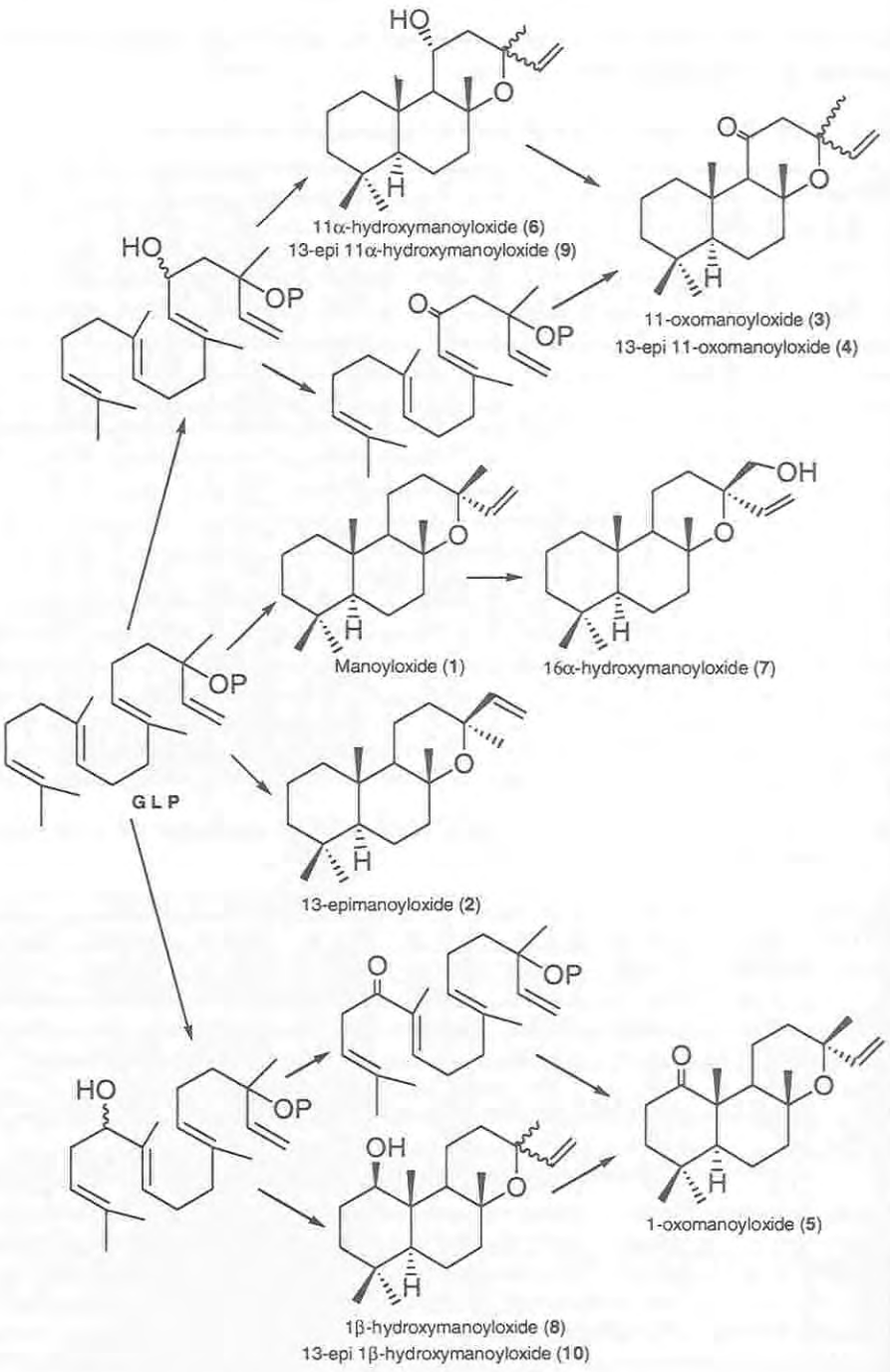

Figure 2. Biosynthetic pathway of manoyloxide and derivatives.

The ^{1}H NMR data of **7** showed the signals of two anisochronous methylene protons of a CH_2OH group (an AB system, δ_H 3.34, δ_H 3.28, J_{AB} = 11.1 Hz). The ^{13}C NMR spectral data of C-1 to C-11 and C-17 to C-20 of **7** were identical to those of **1**, whereas the resonances of C-12 to C-16 led us to place the hydroxyl group at C-16 [7].

The ^{1}H NMR spectrum of **8** showed a geminal proton with a hydroxyl group which appeared at 3.37 ppm as a triplet (J = 7.8 Hz). This chemical shift value compared with the down field shift (3.53 ppm) of carbinolic proton (H-1β) of its 1-α hydroxymanoyloxide epimer, as described in the literature [16], was consistent with chemical shift data of 1-α hydroxy and 1-β hydroxy-4,4(5α) dimethyl cholestane [18]. The ^{13}C NMR spectrum exhibited a signal of Me-20 appearing down field and another one C-1 resonating upfield in comparison with the signals of identical carbons for the epimer 1-α hydroxymanoyloxide [17]. These differences were in good agreement with the data reported in the literature for the isomeric pairs 1-hydroxyandrostane and 1-hydroxycholestane [19]. The only differences observed on the ^{13}C NMR spectral data (C-8, C-9, C-16 and C-17) of **10** compared with those of **8** showed that **10** is a 13-epimanoyloxide derivative. The β (equatorial) configuration of the hydroxyl group at C-1 of **10** is established by comparison of the low value of the chemical shift of C-1 (δ 11.9), as in **8** (δ 11.3).

Table 1 shows that the dichloromethane extract as well as the essential oil [4, 5] of the rhizomes of *K. erecta* is found to contain a large amount of diterpenoid compounds. However the percentage of these components in the essential oils is lower than that from the methylene chloride extract (65% versus 85%). From the extract of the aerial parts, the diterpenoids represent merely 9.4% of the volatiles. Manoyloxide is found to be the most abundant.

The sesquiterpenoid compounds represent more than 55% of the extract of the aerial parts; the total amount of sesquiterpenoids is 8.7% of the extract of the rhizomes. Cyperene is the main component for both samples.

With regard to the quantitative composition, it can be stated that the rhizome extracts were characterised by the diterpenoid compounds while the aerial part extracts are found to contain a large amount of the sesquiterpenoid compounds.

Most of the diterpenoid compounds belong to the labdanic series. Their biosynthetic pathway can be postulated from geranyl-linalyl pyrophosphate (GLP), and involves a succession of allylic oxidations and cyclisations; each step of oxidation, cyclisation, and isomerisation probably requires specific enzyme catalysis (Figure 2). The proposed scheme of the biosynthesis is expected to afford the compounds in the samples of *K. erecta*. But, neither manool nor sclareol, which is the precursor of manoyloxide, has been identified in the extracts of *K. erecta*.

The relatively large quantity of 11-oxo and 11α-hydroxymanoyloxide in the extracts of the rhizomes suggests the presence of forskoline, the natural product isolated from the seed extract of *Coleus forskolii*, a plant from India. It is reported that forskoline has exceptional pharmaceutical properties [19-27]. But the latter component has not been identified in the extract of *K. erecta*.

REFERENCES

1. Gaston, A.; Fotius, G. *Lexique de Noms Vernaculaires des Plantes du Tchad*, Institut d'Élevage et de Médecine Vétérinaires des pays Tropicaux (IEMVPT), Centre Office de la Recherche Scientifique et Technique d'Outre Mer (ORSTOM), Fort-Lami, **1971**; Vol. 1: Noms Scientifiques Noms Vernaculaires, p 110; Vol. 2: Noms Scientifiques Noms Vernaculaires, p 97.

2. Hutchinson, J.; Dalziel, J.M. *The Useful Plants of West Tropical Africa*, Second reprint Royal Botanical Gardens: Kew; **1955**; p 463.
3. Variati, G.L.; Rovesti, P. *Parf. Cosm. Sav.* **1959**, 2-5, 215.
4. Mahmout, Y.; Bessière, J.M.; Dolmazon, R. *J. Agric. Food Chem.* **1993**, 41, 277.
5. Mahmout, Y.; Bessière, J.M.; Dolmazon, R. *Phytochemistry* **1993**, 34, 865.
6. Dolmazon, R.; Albrand, M.; Pollet, P.; Mahmout, Y. *Bull. Soc. Chim. Fr* **1993**, 130, 501.
7. Dolmazon, R.; Albrand, M.; Bessière, J.M.; Mahmout, Y.; Wernerowska, D.; Kolodziejczyk, K. *Phytochemistry* **1995**, 38, 917.
8. Mahmout, Y.; Bessière, J.M.; Dolmazon, R. *Flavour Fragr. J.* **2001**, 16, 100.
9. Joseph-Nathan, P.; Martinez, E.; Santillan, R.L.; Wesener, J.R.; Günther, H. *Org. Magn. Reson.* **1984**, 22, 308.
10. Achenbach, H.; Schwinn, A. *Phytochemistry* **1995**, 38, 1037.
11. Joseph-Nathan, P.; Hernandez, J.D.; Roman, L.U.; Garcia, G.E.; Mendoza, V. *Phytochemistry* **1982**, 21, 669.
12. Akhila, A.; Rani, K.; Thakur, R.S. *Phytochemistry* **1990**, 29, 821.
13. Gabetta, B.; Zini, G.; Danieli, B. *Phytochemistry* **1989**, 28, 859.
14. Enzell, C.R.; Ryhage, R. *Ark. Kemi* **1965**, 23, 367.
15. Werly, F.W.; Nishida, T. The Use of Carbon-13 Nuclear Resonance Spectroscopy in Natural Products in *Progress in Chemistry of Organic Natural Products*, Vol.36, Springer-Verlag: New York; **1979**; pp 55-59.
16. Topcu, G.; Tan, N.; Ulubelen, A.; Sun, D.; Watson, W.H. *Phytochemistry* **1995**, 40, 501.
17. Cambie, R.; Leong, S.H.; Palmer, B.D.; Preston, A.F. *Aust. J. Chem.* **1980**, 33, 155.
18. François, P.; Lablache-Combier, A.; Levissalles, J. *Bull. Soc. Chim. Fr.* **1965**, 2588.
19. Eggert, H.; VanAntwerp, C.L.; Bhacca, N.S.; Djerassi, C. *J. Org. Chem.* **1976**, 41, 71.
20. Seamon, K.B.; Daly, J.W.; Metzger, H.; De Souza, N.J.; Reden, J. *J. Med. Chem.* **1983**, 26, 436.
21. Bhat, S.V.; Dohadwalla, A.N.; Bajwa, B.S.; Dadkar, N.K.; Dornauer, H.; De Souza, N.J. *J. Med. Chem.* **1983**, 26, 486.
22. Caprioli, J.; Sears, M. *The Lancet* **1983**, 958.
23. Lichey, J.; Friedrich, T.; Priesnitz, M.; Biamino, G.; Usinger, P.; Huckauf, H. *The Lancet* **1984**, 167.
24. Seamon, K.B. *Ann. Rep. Med. Chem.* **1984**, 19, 293.
25. Erhardt, P.W. *J. Med. Chem.* **1987**, 30, 231.
26. Hubbard, J.W.; Conway, P.G.; Norstrom, L.C.; Hartman, H.B.; Lebedinsky, Y.; O'Malley, G.J.; Kosley, Jr , R.W. *J. Pharm. Exp. Ther.* **1991**, 256, 621.
27. Laurenza, A.; Seamon, K.B. High-Affinity Binding Sites for [^{3}H] Forskolin, Section I. Adenylyl Cyclase, Ch. 5 in *Methods in Enzymology*, Vol. 195, Johnson, R.A.; Corbin, J.D. (Eds.), Academic Press: New York; **1991**; pp 52-65.