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TRITERPENOIDS OF *CNIDOSCULUS URENS*

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Key Word Index—*Cnidosculus urens*; Euphorbiaceae; β-amyrin cinnamate; germanicol acetate; lupeol acetate; β-amyrin; lupeol; germanicol.

Abstract—The ethanol extract of *Cnidosculus urens* yielded β-amyrin cinnamate and germanicol acetate. Also, using ^{13}C NMR spectroscopy, mixtures of β-amyrin, germanicol and lupeol as well as their acetates were identified.

Preliminary pharmacological studies on the ethanol extract of *Cnidosculus urens* (Euphorbiaceae), a small shrub used in popular medicine [1] showed acetylcholine-like smooth muscle stimulatory effect [2]. The chloroform-soluble part of the ethanol extract, upon further fractionation yielded β-amyrin cinnamate and germanicol acetate along with two apparently homogeneous materials, A and B, which were identified as intimate mixtures of β-amyrin, germanicol and lupeol and their corresponding acetates, respectively, by ^{13}C NMR spectral analysis. This report demonstrates yet another example of the usefulness of ^{13}C NMR spectroscopy in routine identification of relatively common plant products in mixtures which would, otherwise, be a very laborious and wasteful exercise.

The ^{13}C NMR spectrum of β-amyrin cinnamate (1) showed 35 signals for 39 carbons in the molecule. The signals at δ16.9 (two *q*), 23.7 (one *t*, one *q*), 128.0 (two *d*) and 128.8 (two *d*) represent two carbons each. The spectrum showed 30 signals identical to those of the carbons of β-amyrin acetate [3]. However, in place of the acetyl signals, the spectrum showed a singlet at δ166.7 (CO), two doublets at 118.8 (α-CH=) and 144.2 (β-CH=), a singlet a 134.7 (C-1′) and two doublets at 128.0 (C-2′ and C-6′ or C-3′ and C-5′) and 128.2 (C-3′ and C-5′ or C-2′ and C-6′) and a doublet at 130 (C-4′). These signals can be accounted for by the presence of a *trans*-cinnamate moiety instead of an acetate at C-3. The presence of the *trans*-cinnamate moiety is also confirmed by the appearance of two 1H doublets at δ7.66 (J = 16 Hz) and 6.46 (J = 16 Hz) in addition to a 5H multiplet at 7.25–7.55 in the ^{1}H NMR spectrum. Moreover, the alkaline hydrolysis of the compound furnished β-amyrin (3) and cinnamic acid, as expected [4]. Germanicol acetate (4) was identified by comparison of the ^{13}C NMR and other spectral data with those published in the literature [5–7].

The material A, after several crystallizations gave a single spot on a TLC plate but did not show a sharp mp. The molecular weight ($[M]^+$ at m/z 426) of the material corresponded to the formula $C_{30}H_{50}O$. The material A was characterized with the help of ^{13}C NMR spectroscopy. The signals for the olefinic carbons in the ^{13}C NMR spectrum of the pentacyclic triterpenoids are very characteristic and helpful in identifying this type of compound. For example, C-12 and C-13 of the Δ^{12}-oleananes appear at δ121.7 (*d*) and 145.0 (*s*), respectively; the C-18 and C-19 of Δ^{18}-oleananes appear at 142.7 (*s*) and 129.8 (*d*), respectively, and the C-20 and C-29 of $\Delta^{20,29}$-lupanes appear at 150.6 (*s*) and 109.2 (*t*), respectively, which permits the identification of these types of compounds in a mixture with the help of the complete and partial proton noise decoupled ^{13}C NMR spectra. Thus, the olefinic region of the ^{13}C NMR spectrum of the material A showed signals at 150.6 (*s*), 145.2 (*s*), 142.8 (*s*), 129.8 (*d*), 121.8 (*d*) and 109.4 (*t*) which gave an indication

of the probable presence of β-amyrin, germanicol and lupeol (7) in the mixture. The rest of the signals (Table 1) arose out of the remaining carbons of these three above mentioned compounds [3, 5, 8].

The olefinic region of the ^{13}C NMR spectrum of the material B, which also behaved as a homogeneous material (TLC) without a sharp mp, showed signals at δ150.9 (*s*), 145.2 (*s*), 142.7 (*s*), 129.8 (*d*), 121.7 (*d*) and 109.4 (*t*), among others. In addition, the spectrum showed a strong signal at 170.9 (*s*) and a very intense signal at 21.2 (*q*) suggesting the presence of several acetate groups. There is also a very strong signal at 81.0 (*d*) instead of the signal at 79.1 (*d*) in the spectrum of the material A. The former doublet is obviously due to the carbinol carbon at C-3 of the pentacyclic triterpenoids with an acetate group esterified to a β-hydroxyl group. The remaining signals could be assigned to the carbons of the acetates of β-amyrin (2), germanicol (4) and lupeol (6). Therefore, the material B is a mixture of the acetates of β-amyrin, germanicol and lupeol. The confirmation of this comes from the acetylation of the material A which furnished a material having identical R_f value (TLC) and other physical characteristics (solubility, IR) as material B. Also, the ^{13}C NMR spectrum of the acetylated product of material A is practically superimposable on that of material B with only a slight difference in the relative intensities of the signals.

EXPERIMENTAL

All mps are uncorrected. The ^{13}C NMR (20 MHz) spectra were run in a Varian FT80A Spectrometer using $CDCl_3$ as solvent and TMS as internal standard. Column chromatographic separations were performed on glass columns using silica gel (E. Merck).

Plant material. Collected around January 1982 from the campus of the Universidade Federal da Paraíba, João Pessoa and the herbarium sheet is deposited in the LPX Herbarium, Universidade Federal da Paraíba, João Pessoa, PB, Brazil.

Extraction and separation. Dried and powdered *C. urens* (whole plant; 4.6 kg) was extracted with 95% EtOH for 24 hr in a Soxhlet apparatus and the extract was concentrated under red. pres. The dry greenish brown material was partitioned between $CDCl_3$–H_2O (1:1) and the organic layer, after usual treatment gave a residue which was again partitioned between 90% aq. MeOH hexane. The hexane extract, after usual treatment, gave a residue (3.8 g) which was chromatographed using hexane, hexane–C_6H_6, C_6H_6, C_6H_6–$CHCl_3$ mixtures with increasing polarity. Hexane–C_6H_6 (9:1) elution gave a residue (3.61 g) which showed two spots on the TLC plate of which one was fluorescent under a long wave length UV lamp. The material was then subjected to prep. TLC with C_6H_6–$CHCl_3$ (93:7) as developer resulting in an upper fluorescent (blue) band and a lower nonfluorescent band which were treated in the usual manner. The fluorescent band upon several recrystallizations from C_6H_6–hexane yielded β-amyrin cinnamate, $C_{39}H_{56}O_2$ ([M]$^+$ at *m/z* 556), mp 240–242°; UV λ_{max}^{MeOH} nm (log ε): 280 (3.93), 222 (4.16), 216 (4.20) and 202 (3.71); IR ν_{max}^{KBr} cm^{-1}: 3090, 3070, 1715, 1645, 1580, 1455, 1395, 1283, 1210, 1180, 983, 775, 718 and 690; MS *m/z*: 337, 218, 204, 189; ^1H NMR (60 MHz): δ7.66 (1H, *d*, *J* = 16 Hz), 7.25–7.55 (5H, *m*, Ar-H), 6.16 (1H, *d*, *J* = 16 Hz), 5.20 (1H, *m*), 4.72 (1H, *dd*, *J* = 11, 7 Hz, H-3), 1.02 (3H, *s*, Me), 1.00 (3H, *s*, Me), 0.90 (3H, *s*, Me) and 0.89 (3H, *s*, Me). The lower non-fluorescent band, upon usual work up gave germanicol acetate, mp 192–193°, which was characterized by comparison of the physical properties (IR, MS, ^{13}C NMR) with the published data [5–7].

Acetates of β-amyrin, germanicol and lupeol (material B). The mother liquor of the recrystallization of germanicol acetate was found to be a mixture of the acetates of β-amyrin, germanicol and lupeol and it would not be separated by fractional crystallization. It behaved like a homogeneous substance on TLC. It was identified by ^{13}C NMR spectroscopy (Table 1) and no further attempt was made at effecting a separation.

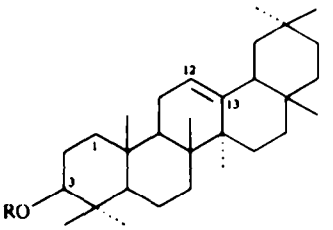
β-Amyrin, germanicol and lupeol (material A). Further elution of the column with C_6H_6–$CHCl_3$ (9:1) gave a material (2.9 g) which was rechromatographed on a smaller column and the hexane–C_6H_6 (7:3) eluate (0.38 g) was recrystallized several times to give a material (material A) which was found to be a mixture (^{13}C NMR) of β-amyrin, germanicol and lupeol. No attempt was made for their separation. Upon acetylation, this material gave an acetate which proved to be identical (TLC, ^{13}C NMR) to the material B.

Acknowledgements—We thank Sra. Maria de Fátima Agra for the collection and identification of the plant material and Prof. Delby Fernandes de Medeiros, Director, Laboratório de Tecnologia Farmacêutica, for his enthusiasm.

Table 1. ^{13}C NMR spectral data (ppm) of the mixtures of β-amyrin (3), germanicol (5) and lupeol (7) and their acetates (2, 4 and 6)

Carbon	3	5	7	2	4	6
1	38.7	38.5	38.8	38.2	38.5†	
2	27.0	27.5	27.3		23.8*	
3		79.1*			81.0*	
4	38.1	39.0	38.8	37.8†	37.9	
5		55.3*		55.4	55.7	55.5
6		18.4*		18.3	18.2†	
7	32.8	36.7	36.6	32.7	32.6†	
8	39.9	41.1†		39.9	41.0	40.9
9	47.8	51.2	50.6	47.7	51.2	50.5
10	37.1	37.3†		36.9	37.4	37.2
11	23.7	21.0†		23.6	21.2	21.0
12	121.8	26.3	25.3	121.7	26.2	25.2
13	145.2	38.8	38.2	145.2	38.7	38.9
14	41.8*			41.8	43.4	42.9
15	28.4	27.5	27.3	28.4	27.2†	
16	26.0	37.8	35.7	26.2	37.8	35.7
17	32.6	34.4	42.8	32.4	34.4	43.0
18	47.4	142.8	48.4	47.4	142.7	48.4
19	46.0	129.8	48.0	46.9	129.8	48.0
20	31.1	32.6	150.2	31.1	32.4	150.9
21	34.8	33.4	29.9	34.8	33.4	29.9
22	37.3	37.4	40.0	37.2	37.4	40.0
23	28.2†		28.0	28.0†		
24	15.5†	15.6		16.7	16.5†	
25	15.5	16.2†		15.6	16.2†	
26	16.9†	16.0		16.7	16.0	16.9
27	26.0	14.6†		26.0	14.5†	
28	27.0	25.3	18.0	27.0	25.3	18.0
29	33.4	31.3	109.4	33.4	31.3	109.4
30	23.6	29.0	19.4	23.6	29.2	19.3
CO	—	—	—	170.9*		
Me	—	—	-	21.2*		

*Signal for three carbons.
†Signal for two carbons.



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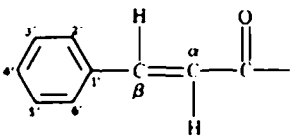

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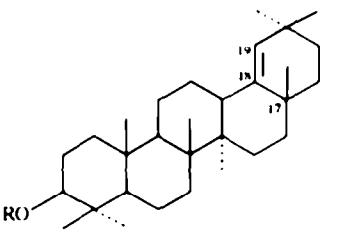
3 R = H



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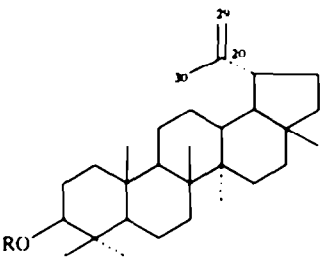
5 R = H



6 R = OAc
7 R = H

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