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STRUCTURE OF ACACIGENIN-B, A NOVEL TRITERPENE ESTER ISOLATED FROM *ACACIA CONCINNA**

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Key Word Index—*Acacia concinna*; Leguminosae; acacigenin-B; ester of acacic acid; novel monoterpene acid; tetrahydrofuranoid.

Abstract—The structure of acacigenin-B, a novel ester genin from the pods of *Acacia concinna* was established from its PMR and ^{13}C NMR spectra. It was identified as the 21-hydroxy ester of acacic acid; the esterifying acid was a hitherto unknown monoterpene acid of tetrahydrofuranoid structure. This appears to be the first report of a higher terpenoid forming an ester with a monoterpene acid

INTRODUCTION

The isolation of a new triterpenoid genin, compound C, from the pods of *Acacia concinna* was reported by us previously [1]. It was shown to be an ester of acacic acid (1). We now report the structure determination of the new genin, now named as acacigenin-B (2).

RESULTS AND DISCUSSION

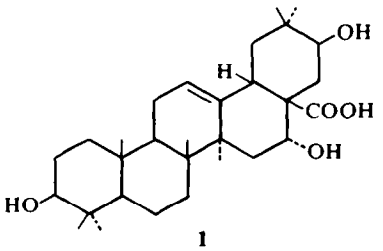
Acacigenin-B crystallized from alcohol as colourless leaflets mp 265–70°, $[\alpha]_D$ + 55°. It analysed for $C_{40}H_{60}O_7$ and showed in its IR spectrum absorptions for —OH (3560 and 3490 cm^{-1}), α:β-unsaturated ester function (1685) and carboxy carbonyl (1710) cm^{-1} respectively.

Hydrolysis of acacigenin-B with alcoholic KOH, followed by careful neutralization at 0° gave acacic acid (1). The aqueous filtrate, after removal of acacic acid, was carefully processed to afford a water soluble acid as a light yellow syrupy mass which could be further purified by crystallization from alcohol as colourless flakes, mp 117°. Thus acacigenin-B is a derivative of acacic acid, one of the hydroxyls of which is in the form of an ester linkage with another unsaturated C_{10} acid. The C_{10} acid was inferred to be a monoterpene acid.

Structure of the monoterpene acid

The monoterpene acid, $C_{10}H_{14}O_3$, M^+ 182, gave a deep yellow colour with TNM. The presence of an α:β-unsaturated acid function was noted from its IR (1685 cm^{-1}) and UV (λ_{max} 218 nm) spectra. The third oxygen was present as an ether as the IR spectrum displayed no —OH stretching but showed a number of strong absorptions in the region 1000–1170 cm^{-1}. Its PMR spectrum showed the following structural features: two methyls on a double bond —C(Me)=C— (δ 1.60, 1.67, *d*, *J* = 7 Hz, 6H), β-proton of an α:β-unsaturated acid —CH=C(—COOH)— (δ 7.18, *m*, 1H), —C(=CH—)—Me (δ 5.55, ill-defined quartet integrating for one proton, which on irradiation reduced to a singlet), —C—O—CH_2 (δ 4.22, 4.38, 4.47, 4.63, AB *q*, *J* = 16 Hz further split by 1 Hz), —CH_2— (δ 2.28, *m*), —CH(—O—CH_2)— (δ 3.78, 3.83, 3.88, 3.93, *dd*, *J* = 10 and 5 Hz, 1H), —COOH (δ 11.2 exchanged with D_2O). These structural features clearly suggested a furanoid structure (3) for the monoterpene acid.

The monoterpene acid formed with diazomethane a methyl ester, an oil, $C_{11}H_{16}O_3$, M^+ 196. This ester, on hydrogenation with Adam's catalyst, gave a mixture of two products (in equal proportions) which could be separated by CC. The hydrogenated methyl ester (4) analysed for a dihydro compound $C_{11}H_{18}O_3$, M^+ 198. Its PMR spectrum (60 MHz) revealed that one of the



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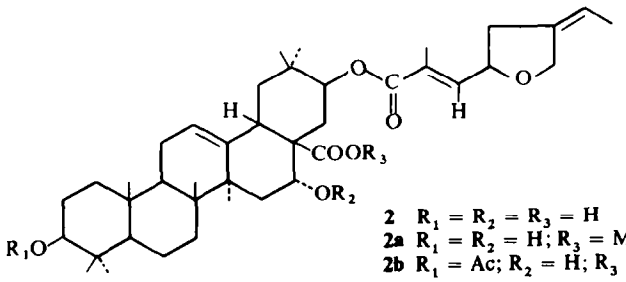
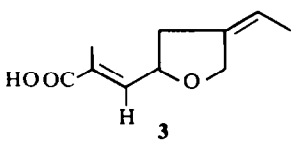


2 $R_1 = R_2 = R_3 = H$
2a $R_1 = R_2 = H$; $R_3 = Me$
2b $R_1 = Ac$; $R_2 = H$; $R_3 = Me$

* Part 3 in the series "Sapogenins of *Acacia concinna*." For Part 2 see (1977) *Indian J. Chem.* **15B**, 7.



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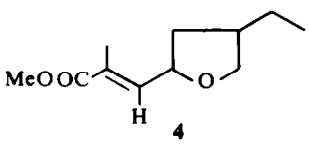

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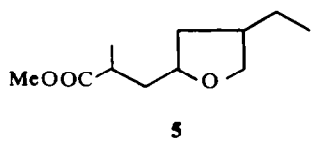

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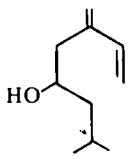

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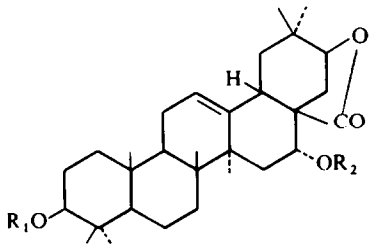


8 $R_1 = R_2 = H$
8a $R_1 = R_2 = Ac$

methyls on a double bond had shifted to δ 0.90 and appeared as a triplet (J = 7 Hz) suggesting that the saturated methyl might have been coupled with the adjacent methylene protons. The vinylic hydrogen at δ 5.6 disappeared. The remaining protons were observed at δ 1.32–1.45 *m* and 2.17 *m* for $-CH_2-$; δ 1.62, 1.66 *d* J = 4 Hz), 3H, for $-C(=CH\underline{H})-C\underline{H}_3$; δ 3.77 *s* for $-COOMe$; δ 3.76 *m* for $\underline{H}C-O-CH_2$; δ 4.43 *m* for $HC-O-C\underline{H}_2$ and δ 7.03 *m* for $\underline{H}C=C(-)-COOMe$.

The hydrogenated ester **5**, $C_{11}H_{20}O_3$, was found to be the completely saturated tetrahydro derivative from its PMR spectrum (100 MHz): δ 0.90 (3H, *t*, J = 7 Hz, $-CH_2-C\underline{H}_3$), 1.20 (2H, *d*, J = 7 Hz, $C\underline{H}_3-CH(-COOCH_3)$) 1.28–1.48 (2H, *m*, $C\underline{H}_2-CH_3$), 2.32 (*m*, CH_2 or $C\underline{H}$), 3.67 (3H, *s*, $-COOC\underline{H}_3$), 3.70 (*m*, $-C-O-C\underline{H}$) and 4.30 (2H, *m*, $-C-O-C\underline{H}_2$).

Mass spectrum of the monoterpene acid

The MS and the fragmentation shown in Scheme 1 for the monoterpene acid clearly confirmed structure (**3**). After loss of the α-side chain, the ion at *m/e* 97 is characteristic of a tetrahydro furanoid [2, 3] skeleton in the molecule. The base peak at *m/e* 85 represents the ion corresponding to the α-side chain.

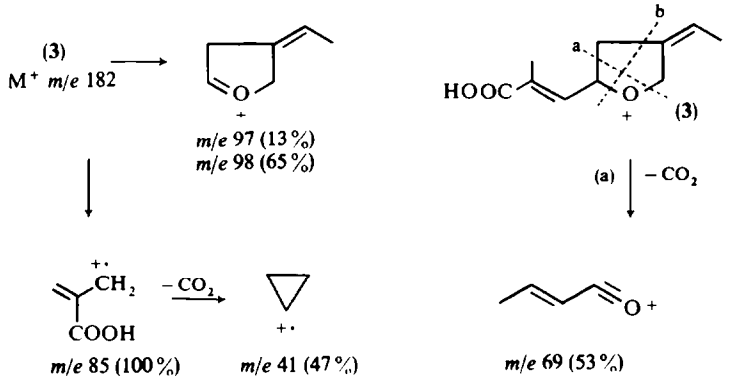
Structure of acacigenin-B

The remaining problem was to decide which of the three hydroxyls of acacigenin-B was linked with the monoterpene acid. Acacigenin-B readily formed an ester (**2a**) and an ester monoacetate (**2b**) indicating that the readily acylable C-3 hydroxyl was free. Either of the remaining C-16 or C-21 hydroxyl groups could have formed the ester linkage. The C-16 hydroxyl being hindered is not likely to be the one involved in such a linkage. Further, had the C-21 hydroxyl been free, it would have behaved as in acacic acid in its reactions such as easy lactonization with the C-17 carboxyl. Acacigenin-B methyl ester did not form the diacetate unlike acacic acid or its lactone (**8**), which readily give **8a**. This behaviour of acacigenin-B might be attributed to the absence of a C-21, C-16 lactone bridge when the C-16 hydroxyl assumes a normal hindered α-axial configuration as in the tetrol [1] (**1**, CH_2OH for COOH) derived from

Table 1. PMR spectrum of acacigenin-B (**2**) in DMSO-d_6/TMS

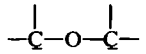
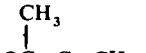
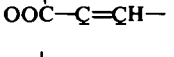
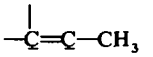
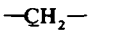
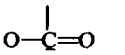
Chemical shift	No. of protons	Assignment
0.66, 0.70, 1.0, 1.33 all *s*	21	7 $C\underline{H}_3$
1.63 *br s*	6	$2{=}C-C\underline{H}_3$*
3.0–3.26 *m*	2	3-O<u>H</u>, 16-O<u>H</u>
3.66 *m*	3	3-H, 16-H, $C-O-C\underline{H}-$*
4.30 *m*	2	$C-O-C\underline{H}_2-$*
5.00 *m*	1	21-<u>H</u>
5.30–5.33 *m*	2	12-<u>H</u>, $=C\underline{H}-CH_3$*
6.93 *m*	1	$-\underline{H}C=C(-)-OO-$*

*Represents the protons belonging to the monoterpene acid unit (**3**).



Scheme 1

Table 2. ^{13}C NMR spectra of acacigenin-B and the monoterpene acid*

Carbon assigned	Acacigenin-B	Carbon assigned	Acacigenin-B	Monoterpene acid
C-1	39.2	C-21	79.4	
C-2	27.6	C-22	37.2	
C-3	78.4	C-23	28.2	
C-4	39.6	C-24	16.4	
C-5	56.4	C-25	14.3	
C-6	18.0	C-26	17.2	
C-7	30.4	C-27	27.6	
C-8	40.2	C-28	181.0	
C-9	48.4	C-29	34.0	
C-10	38.0	C-30	20.5	
C-11	24.0		66.6	68.0
C-12	121.6		79.2	80.0
C-13	143.2		124.0	126.0
C-14	42.2		137.2	139.0
C-15	35.6		139.8	143.0
C-16	74.4		143.2	143.0
C-17	51.2		19.2	
C-18	40.8		20.0	
C-19	47.6		174.5	175.0
C-20	30.0			

* Measurements are given as ppm (δ) downfield from TMS at zero. The spectra were taken on a Varian XL-100 FT instrument at 25 MHz. All assignments are supported by off resonance decoupling.

acacic acid lactone. It can be concluded from the above that acacigenin-B (2) was the C-21 hydroxy monoterpene acid ester of acacic acid (1). The structure of acacigenin-B was further supported by its PMR spectrum (Table 1).

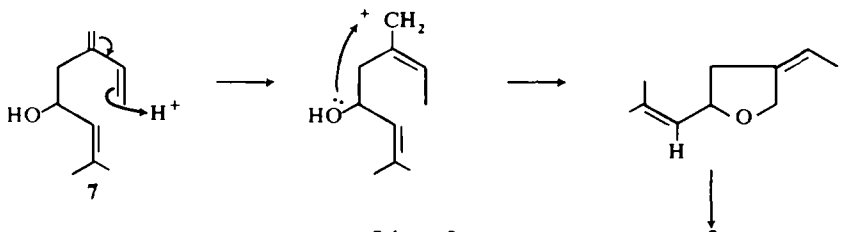
^{13}C NMR spectra of acacigenin-B and the monoterpene acid

The ^{13}C NMR spectra of the pentacyclic triterpenes of the oleanene and ursene groups have been recently studied and all the carbons of the triterpene skeleton have been assigned [4–6]. The ^{13}C NMR spectra of acacigenin-B and the monoterpene acid (Table 2) have now been studied and the assignments made for all the carbons in comparison with the literature values [4–6] lends further support to their assigned structures.

The five signals in acacigenin-B (2) at δ 66.6, 74.4, 78.4, 79.2 and 79.4 were assigned to carbons to which oxygens were attached. The low field signal at δ 79.4 must be that of C-21 with the ester link. The two signals at 74.4 and 78.4 corresponded to carbons C-3 and C-16 to which the hydroxyls were attached. The remaining two signals at δ 66.5 and 79.2 belonged to the two α-carbons of the furan ring of the monoterpene acid. The signals at δ 121.6 and 143.2 were characteristic of the C-12 and C-13 of the oleanene skeleton [4–6]. The three signals at δ 124.0, 137.2 and 139.8 together with signal at 143.2, clearly belonged to the side chain carbons as also noticed in the ^{13}C NMR spectrum of the monoterpene acid. The signals at δ 124.0 and 139.8 corresponded to the α and β carbons of the α:β-unsaturated system. There were two more carbons appearing at δ 137.2 and 143.2 which should obviously be connected by another double bond.

The corresponding signals in the monoterpene acid (3) were noted at δ 126 and 143 for the α,β-carbons of the conjugated acid system and at δ 139 and 143 for the other olefinic carbons. The α-carbons of the furanoid ring were observed at δ 68.0 and 80.0. The signal at δ 32.0 clearly represented the methylene carbon, the other signals of the side chain acid could not be correctly assigned due to their mixing with the background noise.

Triterpenes are known to occur as esters of fatty acids or of aromatic carboxylic acids. The more common acids found are acetic acid [7, 7a], angelic acid [8], tiglic acid [9, 10] and 2,2-dimethyl acrylic acid [11]. Long chain acids like stearic acid [12], palmatic acid [13] and nonadienoic acid [14] and aromatic acids like cinnamic acid [15], *p*-hydroxy cinnamic acid [16] and caffeic acid



Scheme 2

[17] are also known to form esters with triterpene hydroxyls.

The occurrence of mono, sesqui and diterpene carboxylic acids as esters, though biogenetically feasible, has not been reported so far. This report of acacigenin-B (2) esterified with a monoterpene acid (3) seems to be the first of its kind. The monoterpene acid can be regarded to have originated biogenetically from the closely related compounds like isotagetol [18] (6) or more probably from myrcenol (7) [19] as shown in Scheme 2.

EXPERIMENTAL

Mps were uncorr. The NMR spectra were recorded in $CDCl_3$ and the values were reported in δ downfield to TMS.

Acacigenin-B. Acacigenin-B from EtOH as an amorphous powder mp 265–70° $[\alpha]_D$ + 55° (*c*, 1.0). It gave a positive Liebermann–Burchard test for triterpenes. (Found: C, 73.41; H, 9.10. $C_{40}H_{60}O_7$ requires: C, 73.62; H, 9.2%). IR ν_{max} cm^{-1}: 3560, 3480 (—OH), 1718 (COOH), 1685 (α:β-unsaturated ester). UV λ_{max} nm: 220 (log ε 3.03).

Hydrolysis of acacigenin-B and isolation of the monoterpene acid (3). Acacigenin-B (2) (750 mg) was saponified by refluxing with 5% KOH–EtOH (100 ml) on a steam-bath for 4 hr. The EtOH was removed *in vacuo* and H_2O (25 ml) added, cooled to 0° and acidified with ice-cold dil. H_2SO_4. The product was filtered, washed and dried to give a colourless amorphous powder (500 mg) of acacic acid (1), mp and mmp, 280–83°, $[\alpha]_D$ + 68° (*c*, 1.0 in Py). The filtrate, after separating acacic acid, was saturated with $(NH_4)_2SO_4$ and extracted with Et_2O. The Et_2O extract was shaken with $NaHCO_3$ soln which was acidified with cold dil. (1:1) HCl. The soln was again saturated with $(NH_4)_2SO_4$ and re-extracted with Et_2O. The Et_2O layer was dried over dry $MgSO_4$ and evapd to leave a pale yellow coloured semi-solid mass which was crystallized from small quantities of EtOH to give the monoterpene acid (3) as colourless shining plates (150 mg) mp 117° M^+ *m/e* 182. (Found: C, 65.60; H, 7.89. $C_{10}H_{14}O_3$ requires: C, 65.92; H, 7.74%). IR ν_{max}^{Nujol} cm^{-1}: 1685, 1350, 1310, 1270, 1180, 1120, 1050, 970, 880 and 830. UV λ_{max}^{EtOH} nm: 218 (log ε 3.25). The monoterpene acid gave effervescence with $NaHCO_3$ soln, decolourized Br_2 water and dil. $KMnO_4$ soln.

Monoterpene acid methyl ester. The monoterpene acid (100 mg) was dissolved in Et_2O, cooled in ice and treated with ethereal CH_2N_2 at 0° for 2 hr. The soln on evapn gave the methyl ester as a viscous liquid, M^+ *m/e* 196. UV λ_{max}^{EtOH} nm: 220. IR ν_{max}^{Nujol} cm^{-1}: 1695, 1320, 1240, 1120, 1060, 960. (Found: C, 67.20; H, 7.96. $C_{11}H_{16}O_3$ requires: C, 67.35; H, 8.16%).

Hydrogenation of the monoterpene acid methyl ester and isolation of the dihydro (4) *and tetrahydro* (5) *monoterpene acid methyl esters.* The above liquid methyl ester was hydrogenated in EtOH in the presence of Adam's catalyst. The absorption of H_2 (1.4 mol) ceased after 12 hr. The product after removal of EtOH *in vacuo* showed two spots of equal concentration on TLC. The mixture was carefully separated on a small Si gel column into compounds 4 and 5.

Compound 4 (liquid) gave the TNM test for the presence of a double bond. (Found: C, 66.51; H, 8.95. $C_{11}H_{18}O_3$ requires: C, 66.66; H, 9.09%). UV λ_{max}^{EtOH} nm: 220.

Compound 5 (liquid) gave a negative TNM test and was UV transparent. IR ν_{max}^{Nujol} cm^{-1}: 1726 (saturated ester), 1360, 1250, 1035 and 990. (Found: C, 65.92; H, 2.86. $C_{11}H_{20}O_3$ requires: C, 66.01; H, 10.01%).

Methyl ester of acacigenin-B (2a). Acacigenin-B formed the methyl ester with CH_2N_2 at 0°, mp 190–93°, $[\alpha]_D$ + 32.5° (*c*, 1.0 in EtOH). (Found: C, 73.92; H, 9.43. $C_{41}H_{62}O_7$ requires: C, 73.8; H, 9.31%). IR ν_{max}^{Nujol} cm^{-1}: 3580, 3480 (OH), 1735 (COOMe), 1685 (α:β-unsaturated ester) 1240, 1100, 1030, 990, 920, 860, 760.

Methyl ester acetate of acacigenin-B (2b). Acacigenin-B methyl ester (2a) (200 mg) gave an acetate with Ac_2O (15 ml) and Py (15 ml) when heated on a steam-bath for 3 hr. The acetate crystallized from EtOH mp 175–78°, $[\alpha]_D$ + 65° (*c*, 1.0 in EtOH). (Found: C, 73.15; H, 9.18. $C_{43}H_{64}O_8$ requires: C, 72.88; H, 9.04%). UV λ_{max}^{EtOH} nm: 219 (log ε 3.21). IR ν_{max}^{Nujol}: 3610 (16-OH), 1740–1735 (COOMe and OCOOMe), 1690 (α:β-unsaturated ester), 1275, 1240, 1180, 1100, 1080, 990, 920, 850, 765, 735 and 715.

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REFERENCES

1. Anjaneyulu, A. S. R., Bapuji, M., Gapala Rao, M., Row, L. R., Sastry, C. S. P., Sree, A. and Subrahmanyam, C. (1977) *Indian J. Chem.* **15B**, 1.
2. Porter, Q. N. and Baldas, J. (1971) *Mass Spectrometry of Hetero-Cyclic Compounds*, p. 39. Wiley-Interscience, New York.
3. Oholoff, G., Schulte-Elte, K. H. and Wilhalm, B. (1964) *Helv. Chim. Acta* **47**, 602.
4. Tori, K., Seo, S., Shimako, A. and Tomita, Y. (1974) *Tetrahedron Letters* 4227.
5. Seo, S., Tomita, Y. and Tori, K. (1975) *Tetrahedron Letters* 7.
6. Seo, S., Tomita, Y. and Tori, K. (1975) *J. Chem. Soc. Chem. Commun.* 954.
7. Anjaneyulu, V. and Row, L. R. (1969) *Curr. Sci.* **37**, 156.

7a. Wulff, G. and Tschesche, R. (1969) *Tetrahedron* **25**, 515.

8. Errington, S. G., White, D. E. and Fuller, M. W. (1967) *Tetrahedron Letters* 1289.
9. Anderson, L. A. P., de Kock, W. T. and Enslin, P. R. (1961) *J. S. Afr. Chem. Inst.* **14**, 58.
10. Barua, A. K., Dutta, S. P. and Das, B. C. (1968) *Tetrahedron* **24**, 1113.
11. Hiller, K., Linzer, B., Pfeifer, S., Tokes, L. and Murphy, J. (1968) *Pharmazie* **23**, 376.
12. Pai, P. P. and Kulkarni, G. H. (1977) *Curr. Sci.* **46**, 261.
13. Awasthi, Y. C. and Mitra, C. R. (1968) *Phytochemistry* **7**, 637.
14. Kupchan, S. M., Takasugi, M., Smith, R. M. and Steyn. P. S. (1971) *J. Org. Chem.* **36**, 1972.
15. Ogino, T., Hayaska, T., Ito, S. and Takahashi, T. (1968) *Chem. Pharm. Bull. (Tokyo)* **16**, 1846.
16. Yasue, M., Sakakibara, J. and Kaiya, T. (1971) *Yakugaku Zasshi* **91**, 1200.
17. Inubushi, Y., Harayama, J., Hibino, T. and Somanathan, R. (1970) *J. Chem. Soc. D.* **17**, 1118.
18. Silverstein, R. M., Rodin, J. O., Wood, D. L. and Browne, L. E. (1966) *Tetrahedron* **22**, 1929.
19. Devon, T. K. and Scott, A. I. (1972) *Handbook of Naturally Occurring Compounds*, Vol. 2. Academic Press, New York.