Phytochemistry, Vol 23, No 10, pp 2251–2255, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

3-OXO-FRIEDELAN-20α-OIC ACID FROM *GYMNOSPORIA EMARGINATA*

PARIMI ATCHUTA RAMAIAH, (MRS) PARIMI UMA DEVI, FELIX FROLOW and DAVID LAVIE

Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel

(*Received* 5 *December* 1983)

Key Word Index—*Gymnosporia emarginata*, Celastraceae, β-amyrin, D A-friedo-oleananes, friedelin, 3-oxo-friedelan-20α-oic acid, maytenonic acid, polpunonic acid, sitosterol

Abstract—3-Oxo-friedelan-20α-oic acid, named as maytenonic (polpunonic) acid, has been isolated along with β-amyrin and sitosterol from *Gymnosporia emarginata* ^{1}H and ^{13}C NMR signals have been assigned for the structure X-ray analysis of the single crystal confirmed that the carboxylic acid is α-oriented at C-20 and the D/E rings of this D A-friedo-oleanane are in chair–chair conformation ^{13}C NMR data of the present compound also enabled the assignment of the C-20α- and β-methyl carbons in friedelin

INTRODUCTION

Gymnosporia emarginata Laws [1] (Celastraceae) is a thorny shrub with pale leaves, and frequently bearing flowers, which is available in the area of Nuzvid (Andhra Pradesh, India) The plant is called '*Danti*' in the local language (Telugu) and is used occasionally for fencing Tincture extracts of the bark are used in local medicine Several *Gymnosporia* species have been reported for their medicinal properties and chemical constituents [2, 3] Recently, a chemical examination of this plant from Sri Lanka revealed the presence of 3-oxo-lup-20(29)-en-30-al and two of its derivatives [3] The present chemical study of this plant, which was collected from the Nuzvid area, yielded a triterpene acid, 3-oxo-friedelan-20α-oic acid, named maytenonic (polpunonic) acid (**2a**) [4, 5] along with β-amyrin and sitosterol Maytenonic acid (**2a**), mp 262°, $[\alpha]_D - 32°$ (MeOH) was first reported [4] from *Maytenus senegalensis* (Celastraceae) The stereochemistry of the carboxylic acid at C-20 was not settled, and based on mass spectral fragmentation data, its orientation was proposed as *syn* with the 27-Me Further, it was found that the extracts and fractions from the plant had reproducible activity against the 9 KB carcinoma of the nasopharynx in cell cultures, as well as the L-1210 leukemia and PS leukemia tumor systems in mice [4] Later this compound was also isolated [5] from the roots of *Plenckia polpunea* (Hippocrateaceae) and named polpunonic acid, mp 275–276°, which was reported to show antibacterial and antimitotic activity The stereochemistry of the carboxylic acid at C-20 was described as being α by subjecting its 3-hydroxy-29-carboxylic acid methyl ester to the friedelane-oleanane rearrangement to obtain the 18,29-lactone [5]

Recently, the structure of maytenfoliol, a D A-friedo-oleanane (17β,20β-dihydroxymethyl-3-oxo-friedelan) was established by X-ray and its antileukemic activity was reported In this case the rings A–C are in chair forms, whereas in the *cis* fused D/E rings, ring E is close to a boat form while ring D is twisted from a boat towards a stretched 1,3-diplanar form [6] It was also shown earlier by X-ray studies for D A-friedo-oleananes, that the A–C rings are in chair forms and the *cis*-fused D/E rings may be either in the folded chair–chair [7, 8] or in the stretched boat–boat [9–12] conformations It was suggested by ^{1}H NMR studies using LIS and INDOR techniques that the *cis* fused D/E rings of friedelin (**1**) in solution assume a boat–boat conformation [13] ^{13}C NMR LIS values for a few D A-friedo-oleananes in solution had been recently described as supporting a boat–boat rather than a chair–chair conformation for the *cis* fused D and E rings [14]

Similarly, if the *cis* fused D/E rings of the present compounds (**2a**) and (**2b**) are in boat–boat conformation, the ^{1}H and ^{13}C NMR signal assignments will be more suggestive of a 20β- rather than the reported 20α-oriented carboxy group This controversy for the fixation of the stereochemistry at C-20 has now been clarified by carrying out a single crystal X-ray analysis of compound **2b** which showed that the *cis* fused D/E rings are in chair–chair conformation along with A–C rings being all in chair forms as shown in Fig 1 Now, the ^{1}H and ^{13}C NMR signals have been readily assigned for compounds **2a** and **2b**, and this has finally corroborated the assignments of the α and β-methyl carbons at C-20 in friedelin (**1**)

RESULTS AND DISCUSSION

Compound **2a** analysed for $C_{30}H_{48}O_3$, $[M]^+$ at *m/z* 456, mp 261–262°, $[\alpha]_D - 41\,6°$ The presence of the six membered ring ketone (1700 cm^{-1}) and the carboxylic acid (3300–2500 broad and 1710 cm^{-1}) were seen in the IR spectrum, as well as the signals, respectively, at δ213 3 and 184 9 in the ^{13}C NMR spectra It gave a monomethyl ester **2b** with diazomethane, which analysed for $C_{31}H_{50}O_3$ The methyl ester showed the ester carbonyl (1720 cm^{-1}) in the IR spectrum and a singlet at δ3 64 for the carbomethoxy group in the ^{1}H NMR spectrum

Analysis of the ^{1}H NMR spectrum of **2a** showed five singlets and one doublet (Table 1), totally integrating for seven methyl groups One of the signals of the methyl doublet (δ0 87, $J = 6$ Hz) was overlapped by that at δ0 88 Upon irradiation at the C-4 secondary methyl signal, the 4α-H_{ax} quartet collapsed to a sharp singlet at

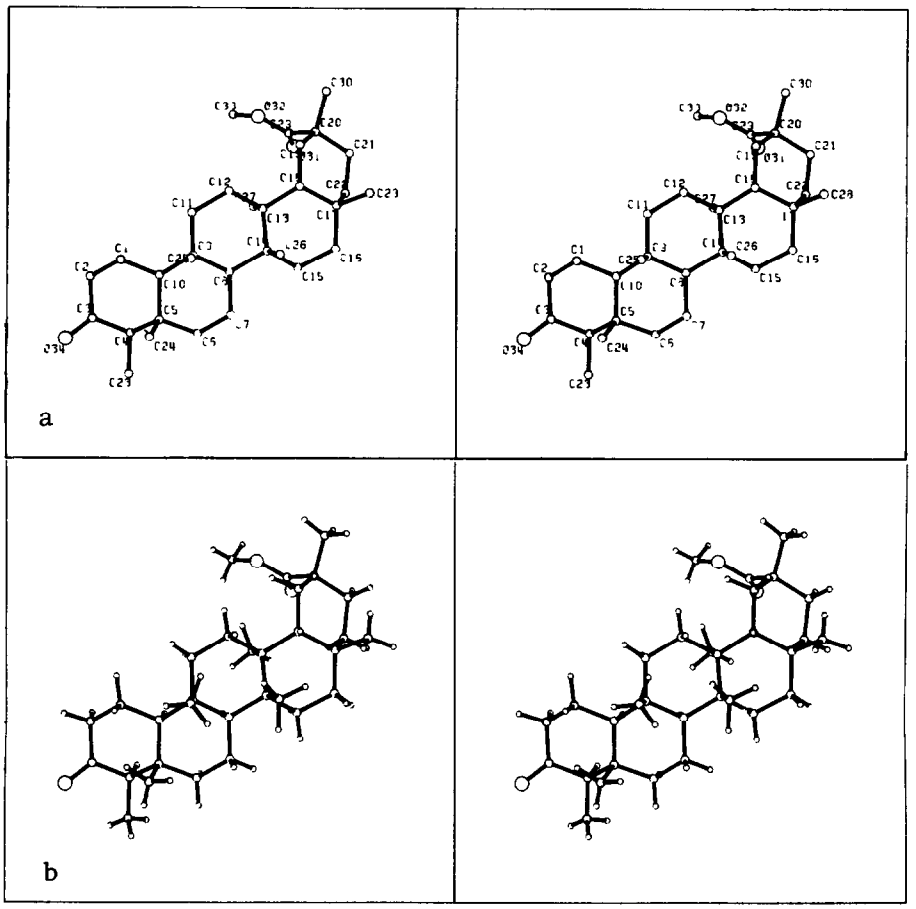
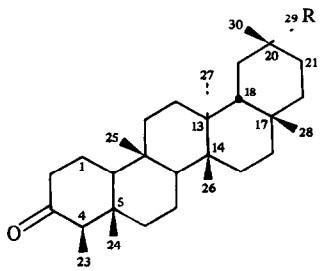


Fig 1 Stereoscopic view of maytenonic (polpunonic) acid methyl ester **2b** (a) with numbering, (b) with hydrogens

δ2 21 Reciprocal irradiation at this point, resulted in the conversion of the C-4 methyl from a doublet to a singlet, thereby enabling the assignment of the chemical shifts of 4α-H_{ax} and the 23β-Me (*eq* at C-4) groups Since this compound (**2a**) belongs to a D A-friedo-oleanane series of triterpenes, a comparison was made with the ^{1}H NMR spectral values of friedelin (**1**) [15] and the same chemical shifts were found for the C-23, C-24 and C-25 methyl groups The singlet at δ1 26, the lowest of the methyl region, was assigned to that methyl adjacent to the carboxylic acid at C-20, and the signals for the remaining C-26, C-27 and C-28 methyls were deduced by using the chemical shift differences observed between the acid and the ester (Table 1) The C-23 and C-24 methyl groups in **2a** and **2b** were found at the same positions, but the C-25 methyl group shifted only slightly upfield, by 0 02 ppm The signal for the methyl group adjacent to the carboxylic acid moved to δ1 18 in **2b** and this shift of + 0 08 ppm is believed to be due to the absence of the deshielding and the lowering of the electron withdrawing effect of the acid by ester formation The remaining signal positions for the C-26, C-27 and C-28 methyl groups were selected by comparing the shifts observed for pairs of close values between **2a** and **2b** The best assignments were those for the C-27 methyl group 1 00 for **2a** and 0 87 for **2b**, the difference being + 0 13 ppm Such an effect can be attributed to the position of this methyl group which is under the direct influence of the carboxy-group, both being on the same side of the molecule (see Fig 1) The remaining pair of signals were for the C-26 and C-28 methyl groups, the assignment of the latter being deduced from its close value to the appropriate methyl group of friedelin (**1**) [15], as well as from a correlation of the residual ^{13}C–H coupling constant observed in the CMR SFORD spectra of **2a** and **2b**



	R
1	Me
2a	COOH
2b	COOMe

One should keep in mind, however, that should the D/E rings have been in a boat–boat conformation, then the carboxy group would have been β-oriented at C-20 in order to influence the proximate methyl group which should then be the C-28 methyl group to account for the above shift difference (+ 0 13 ppm) This conjecture is ruled out as seen from the X-ray structure of **2b**

Table 1 ^{1}H NMR signals of relevant protons in compounds **2a** and **2b**

Compound	4α-H	Me groups 23β	24β	25β	26β	27α	28β	29α	30β	29α-CO_2Me
1*		0 88 (*d*)	0 73	0 88	1 01	1 05	1 18	0 97	1 01	—
2a	2 21 (*q*,7)	0 87 (*d*,6)	0 71	0 88	0 88	1 00	1 09	—	1 26	—
2b	2 23 (*q*,7)	0 87 (*d*,6 5)	0 71	0 86	0 86	0 87	1 07	—	1 18	3 64
$\Delta = \delta$**2a** $- \delta$**2b**	−0 02	0 00	0 00	+0 02	+0 02	+0 13	+0 02	—	+0 08	—

Chemical shifts are in δ units measured in $CDCl_3$ Coupling constants (Hz) are in parentheses Except for the C-23 methyl group all methyl groups are singlets

*Values are taken from ref [15]

In Fig 1, although ring E is definitely in a chair conformation, there seems to be a very slight tilt of C-20 due to steric repulsion between the C-27 methyl group and the methyl ester Furthermore, it can be seen that all the atoms of the methyl ester group locate themselves in a plane which puts them as far as possible from the bulky C-27 methyl group Upon careful observation of these groupings, one sees very well that the C-27 methyl group is located under the carbonyl shielding cone of the ester If this is also true in solution, it supports well the shielding effect of the carbonyl of the methyl ester resulting in the value of δ0 87 for the C-27 methyl group compared to 1 00 in the carboxylic acid, in which the delocalization of electrons reduces the carbonyl character of the carboxylic acid

The proton-noise decoupled ^{13}C NMR spectra of compounds **2a** and **2b** showed signals for all the carbon atoms, as given in Table 2 Since these compounds belong to a D A-friedo-oleanane pentacyclic triterpene series, the ^{13}C NMR data available for this class of compounds contributed to the present assignment

The ^{13}C NMR values for all the thirty carbons of friedelin (**1**) were first assigned by Beierbeck *et al* [16] Subsequently, without being aware of these values, by using various NMR techniques, including lanthanide induced shift data on several friedelane keto-derivatives, the chemical shift values for most of the carbons in friedelin (**1**) were differently assigned by Gunatilaka *et al* [17] The assignments were, however, found subsequently to be inconsistent and the same values were reassigned by Patra *et al* [18] These authors were also not aware of the above first assignments [16] given for friedelin The reassigned values of ref [18] were then accepted by the Sri Lanka team [17], which also used them for additional assignments of some D A-friedo-oleananes [14]

Our careful analysis of the chemical shift values for friedelin given in both refs [16] and [18] were found to be mostly the same, except for the mutual differences in assigning the secondary C-2 and quaternary C-5, as well as the C-21 and C-22 methylene and C-29 and C-30 methyl carbons in ring E The SFORD spectra of **2a** and **2b** showed a triplet for the secondary C-2 and a singlet for the quaternary C-5 observed at 41 5 and 42 1 ppm, respectively, and therefore these values were assigned for friedelin which agree with ref [18] The values assigned for C-21 and C-22 were found to be also more appropriate in ref [18] than in ref [16], since the analysis was based on the ^{13}C NMR data available for C-21 and C-22 keto compounds of D A-friedo-oleananes [14, 18] ^{13}C NMR values of our 20α-oriented carboxy ester group in compound **2b**, as evidenced from the X-ray structure, support the assignments for C-29 and C-30 methyl carbons in friedelin given in ref [16] and not as in ref [18] Hence, in our view the more appropriate values for friedelin (**1**) are as now presented in Table 2 and used to assign the ^{13}C values for **2a** and **2b** as described below

Since the carboxy group at C-20 does not influence the carbon chemical shifts of the A and B rings and their substituents, the values for the C-23, C-24 and C-25 methyls and the ring carbons (except C-8) in **2a** and **2b** were found respectively to be the same as in friedelin (**1**) with a variation of ± 0 3 ppm Similarly, the β-oriented C-26 and C-28 methyls were not influenced and found respectively at the same positions as in **1**

The presence of the carboxy group caused a deshielding effect on C-20 and shielding on the adjacent carbons 19, 21 and 30, when compared to friedelin (**1**) Similar types of effects were reported [19] in the case of the two C-20 epimeric carboxy methyl esters of β-amyrin, namely 3β-acetoxy-11-desoxo-glycyrrhetic-20β_{ax}-acid and -liquiritic-20α_{eq}-acid when compared to β-amyrin Even though these two epimeric carboxy acid esters with the same *cis* fused D/E rings and chair–chair conformation were distinguishable from each other due to the difference in the chemical shifts of the above four carbons, a direct comparison of these differences could not establish the stereochemistry of the carboxy group in **2a** and **2b**, when compared to friedelin This is due to the difference in conformation at the 20-carbon existing between the oleanane and friedelane skeletons being 20α-equatorial in the former [19] and 20α-axial in the latter The C-27 methyl group in **2a** and **2b** which is *syn* with the 20α_{ax}-carboxy group was found to be shielded by +4 0 and +4 2 ppm respectively, when compared to friedelin This shielding effect may be attributed either to the steric effect and mutual interaction with the carboxy group, or to a possible boat–boat conformation in friedelin The three carbons, 8, 15 and 22 were respectively shielded by +2 4, +2 2 and +2 5 ppm in compound **2a** and +2 7, +2 4 and +2 6 ppm in **2b**, again when compared to friedelin These shielding effects might have been generated by the newly formed γ-effects, as well as γ-gauche interactions [17, 19, 20] due to the effects mentioned above All the other signals are close to the friedelin (**1**) values

Table 2 ^{13}C NMR spectral data of compounds **1**, **2a** and **2b**

Carbon	1*	2a	2b	Carbon	1*	2a	2b
1	22 3	22 3	22 3	17	30 0	30 2	30 2
2	41 5	41 5	41 5	18	42 8	44 3	44 6
3	213 0	213 3	213 0	19	35 4	29 5	29 2
4	58 2	58 3	58 2	20	28 1	40 5	40 6
5	42 1	42 1	42 1	21	32 8	29 5	29 6
6	41 3	41 4	41 3	22	39 2	36 7	36 6
7	18 2	18 3	18 2	23	6 8	6 8	6 8
8	53 1	50 7	50 4	24	14 6	14 6	14 6
9	37 4	37 5	37 5	25	17 9	18 1	17 5
10	59 5	59 9	59 6	26	18 6	18 4	18 5
11	35 6	35 3	35 2	27	20 3	16 3	16 1
12	30 5	30 3	30 5	28	32 1	31 8	31 9
13	38 3†	39 2†	39 3†	29	31 8	184 9	179 3
14	39 7†	39 3†	39 4†	30	35 0	31 5	31 9
15	32 4	30 2	30 0	$COO\underline{C}H_3$	—	—	51 5
16	36 0	36 2	36 2				

*Values are taken from refs [14, 16 and 18] with appropriate alterations
†Values are interchangeable vertically

The mass spectrum of compound **2a** showed the characteristic fragmentations for friedelane triterpenes [21, 22], and these are given in the Experimental section From *Catha cassinoides* [23] 20β-hydroxymethyl friedelin had been isolated and from its acetate an X-ray structure analysis has been carried out It was found that the *cis* fused D and E rings are in twist and perfect boat forms, respectively It can be assumed therefore that the corresponding β-oriented acid may be in the same conformation This conformation is quite different for this area of the molecule than our 20α-axially oriented carboxy ester of friedelin which has a chair-chair conformation (Fig 1)

The present results show that the *Gymnosporia emarginata* bark is a new source for the biologically active compound maytenonic (polpunonic) acid (**2a**) whose structure is unequivocally established

EXPERIMENTAL

Gymnosporia emarginata plant material was collected 5 km south-west of Nuzvid, India Mps were measured on a Fischer–Johns apparatus and are uncorr IR spectra were recorded on a Perkin–Elmer 467 grating spectrophotometer ^1H NMR and ^{13}C NMR spectra (broad band and SFORD) were determined with Bruker WH270 (270 MHz) and WH90 (operating at 22 63 MHz) instruments, respectively, for $CDCl_3$ solns with TMS as internal standard Analytical TLC was carried out using chromatoplates (50 × 75 mm, silica gel F_{254}) TLC plates were monitored under UV lamp and also by spraying with 5% conc H_2SO_4 in MeOH and heated to 120° MS were determined under the direction of Dr Z Zaretskii, and microanalyses were carried out by Mr R Heller of this Institute X-ray single-crystal analysis was made using three-dimensional intensity data, collected on a computer controlled Enraf-Nonius (CAD-4 diffractometer [λ(Cu-K$\bar{\alpha}$) = 1 5405 Å] by ω-2θ technique (θ < 70°) at room temp

Examination of leaves isolation of β-amyrin The air dried leaves (0 5 kg) were powdered and successively extracted in a Soxhlet with hexane (3 l) and MeOH (3 l) The former was concd under red pres to give a green gummy residue (30 g) out of which 5 g were taken and crystallized several times from hot MeOH to yield 4 g of colourless needle shaped crystals, single pink spot upon TLC, R_f 0 42 (hexane–EtOAc, 4 1), it gave a positive Liebermann–Burchard test (pink colour for triterpenes, mp 194–196°, $[\alpha]_D$ + 86 7° (*c* 0 93, $CHCl_3$) which was identified as β-amyrin (lit [24] mp 197°) It gave a monoacetate with Ac_2O and pyridine at room temp, crystallized from hexane, mp 240°, $[\alpha]_D$ + 81 2° (*c* 0 81, $CHCl_3$) Oxidation with CrO_3 in pyridine yielded the 3-monoketone crystallized from MeOH, mp 171–172°, $[\alpha]_D$ + 106 4° (*c* 0 43, $CHCl_3$ [lit [24] mp 177–178°) The mp, $[\alpha]_D$, IR, ^1H NMR and MS data of the parent compound, its acetate and the 3-keto derivative are in complete agreement with β-amyrin and its corresponding derivatives [24–26] Also the ^{13}C NMR data of the compound and its acetate were found identical with the reported values [27] for β-amyrin and its acetate

Examination of bark The air dried bark (1 kg) was powdered and extracted with $CHCl_3$ (3 l) in a Soxhlet The extract was concd under vacuum to a brown syrup and dissolved in hexane (400 ml) Slowly a pale brown coloured substance separated which was collected by filtration Concn of the mother liquors provided additional substance A total of 6 g was obtained, showing three major spots on TLC (hexane–EtOAc, 4 1) The mixture was adsorbed on silica gel (20 g) and fractionated over a chromatography column (6 × 90 cm), packed with silica gel (250 g) and eluted with solvent mixtures of hexane and EtOAc in the following proportions 9 1, 8 2, 6 4 and 50 ml fractions were collected by TLC monitoring

The hexane fractions gave oily substances of no further interest Some of the hexane–EtOAc (9 1) fractions gave a single compound which crystallized from MeOH (yield 600 mg), mp 194–196°, which was identified as β-amyrin by comparing with the sample isolated from the above leaf extract The latter fractions from hexane–EtOAc (9 1), afforded the second compound which crystallized from MeOH (yield 200 mg), mp 136–137°, $[\alpha]_D$ − 35° ($CHCl_3$), which was identified as sitosterol when compared with an authentic sample, and also through its acetate mmp, TLC (R_f 0 27, hexane–EtOAc, 4 1, green spot), IR, ^1H NMR [28] and ^{13}C NMR [29] spectra The fractions from hexane–EtOAc (8 2), afforded the third compound **2a** (400 mg)

3-Oxo-friedelan-20α-carboxylic acid (**2a**) Mp 261–262°

(MeOH), $[\alpha]_D$ −41·6° (*c* 1·5, $CHCl_3$), TLC R_f 0·48 (hexane–EtOAc, 3·2, pink spot), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3300, 2500, 2940, 2860, 2680, 1710 and 1700 MS (LR) *m/z* (rel int.) 456 (95·6) $[M]^+$, 441 (33·7) $[M-CH_3]^+$, 410 (16·2) $[M-HCOOH]^+$, 395 (11·5) $[M-HCOOH-CH_3]^+$, 371 (19·4) $[M-85$ (C_5H_9O, cleavage of ring A + H)$]^+$, 302 (14) $[M-154$ ($C_9H_{14}O_2$, cleavage of rings D, E)$]^+$, 273 (100) $[M-183$ ($C_{11}H_{19}O_2$, cleavage of rings D,E + H)$]^+$, 250 (81) $[M-206$ ($C_{14}H_{22}O$, cleavage of rings A,B,C)$]^+$, 235 (47) $[M-221$ ($C_{15}H_{25}O$, cleavage of rings A,B,C + H)$]^+$, 221 (26) [cleavage of rings A,B,C + H]$^+$, 155 (90) [$C_9H_{15}O_2$, cleavage of rings D,E + H]$^+$ MS (HR) *m/z* (rel int.) 441·3325 (10·7) [$C_{29}H_{45}O_3$]$^+$, 410·3514 (6·3) [$C_{29}H_{46}O$]$^+$, 273·2214 (52·8) [$C_{19}H_{29}O$]$^+$, 250·1928 (26·3) [$C_{16}H_{26}O_2$]$^+$, 235·1691 (14·6) [$C_{15}H_{23}O_2$]$^+$, 155·1062 (77·3) [$C_9H_{15}O_2$]$^+$, 109·0999 (100·0) [C_8H_{13}]$^+$ (Found: C, 78·84; H, 10·61. $C_{30}H_{48}O_3$ requires C, 78·89; H, 10·59%; MW 456·7.)

3-Oxo-friedelan-20α-carboxymethyl ester (**2b**). Compound **2a** (100 mg) dissolved in Et_2O (100 ml) was cooled to 0° and an ice cooled Et_2O soln of CH_2N_2 was added. After 24 hr in the cold, the reaction mixture was worked up by the usual procedure and purified by passing through a small chromatographic column packed with silica gel. Methyl ester **2b** crystallized from EtOH, mp 225–226°, TLC R_f 0·71 (hexane–EtOAc, 3·2), pink coloured spot, $[\alpha]_D$ −35° (*c* 1·0, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 2940, 2860, 1720 and 1700 (Found: C, 78·94; H, 10·68. $C_{31}H_{50}O_3$ requires C, 79·09; H, 10·62%; MW 470·7.) Crystal data: orthorhombic $a = 6·801$ (1), $b = 16·108$ (2), $c = 24·658$ (3) Å, space group $P2_12_12_1$, and $Z = 4$. The structure was solved by direct methods and refined using 2737 unique reflections with $F_\phi > 3\sigma(F_\phi)$. The nonhydrogen atoms were treated with anisotropic temperature factors. All hydrogens were found from a difference Fourier map and refined without constraints (C–H bonds ranged between 1·20 and 0·82 Å) with overall isotropic temperature factor (U = 0·08 $Å^2$). Block diagonal least-squares refinement converged to $R = 0·04$. The final difference Fourier map revealed only randomly distributed electron density (maximum peak of 0·3 $eÅ^{-3}$).

Acknowledgements—We thank Mr D. Krishnamurthy and Mr S. L. John, Department of Botany, Dharma Appa Rao College, Nuzvid, India, for identification of the plant material, and Dr H. E. Gottlieb of this Institute for fruitful discussions. We are grateful to the Deutscher Akademischer Austauschdienst for a scholarship to P. A. R. (on leave of absence from Andhra University, Waltair, India). P. A. R. wishes to thank UGC, India for a small research grant to partly complete this work at A. U. P. G. Extension Center, Nuzvid.

REFERENCES

1 Gamble, J. S. (1967) *Flora of the Presidency of Madras*, Vol. I, p. 151. Botanical Survey of India, Calcutta.
2 Watt, J. M. and Breyer-Brandwijk, M. G. (1962) *Medicinal and Poisonous Plants of Southern and Eastern Africa*, p. 183. E. & S. Livingstone, London.
3 Dharmassree, B. T. W., Vijaya Kumar and Sultanbawa, M. U. S. (1981) *J. Chem. Soc. Perkin Trans.* 1, 2724.
4 Abraham, D. J., Trojanek, J., Munzing, H. P., Fong, H. H. S. and Farnsworth, N. R. (1971) *J. Pharm. Sci.* **60**, 1085.
5 Monache, F. D., de Mello, J. F., Marini-Bettolo, G. V., de Lima, O. G. and d'Albuquerque, I. L. (1972) *Gazz. Chim. Ital.* **102**, 636.
6 Nozaki, H., Suzuki, H., Lee, K. H. and McPhail, A. T. (1982) *J. Chem. Soc. Chem. Commun.* 1048.
7 Corey, E. J. and Ursprung, J. J. (1956) *J. Am. Chem. Soc.* **78**, 5041.
8 Kikuchi, T., Niwa, M. and Masaki, N. (1972) *Tetrahedron Letters* 5249.
9 Masaki, N., Niwa, M. and Kikuchi, T. (1975) *J. Chem. Soc. Perkin Trans.* 2, 610.
10 Monache, F. D., Marini-Bettolo, G. B., Pompani, M., de Mello, J. F., King, T. J. and Thomson, R. H. (1979) *J. Chem. Soc. Perkin Trans.* 1, 2649.
11 Rogers, D., Phillips, F. L. Joshi, B. S. and Viswanathan, N. (1980) *J. Chem. Soc. Chem. Commun.* 1048.
12 Laing, M., Burke-Laing, M. E., Bartho, R. and Weeks, C. M. (1977) *Tetrahedron Letters* 3839.
13 Kikuchi, T., Shingu, T., Niwa, M. and Yokoi, T. (1973) *Chem. Pharm. Bull. Jpn.* **21**, 1396.
14 Gunatilaka, A. A. L., Nanayakkara, N. P. D. and Wazeer, M. I. M. (1983) *Phytochemistry* **22**, 991.
15 Kikuchi, T., Yokoi, T., Niwa, M. and Shingu, T. (1980) *Chem. Pharm. Bull. Jpn.* **28**, 2014.
16 Beierbeck, H., Saunders, J. K. and ApSimon, J. W. (1977) *Can. J. Chem.* **55**, 2813.
17 Gunatilaka, A. A. L., Nanayakkara, N. P. D., Sultanbawa, M. U. S. and Wazeer, M. I. M. (1980) *Org. Magn. Reson.* **14**, 415.
18 Patra, A., Mukhopadhyay, A. K. and Mitra, A. K. (1981) *Org. Magn. Reson.* **17**, 166.
19 Ricca, G. S., Danieli, B., Palmisano, G., Duddeck, H. and Elgamal, M. H. A. (1978) *Org. Magn. Reson.* **11**, 163.
20 Dalling, D. K. and Grant, D. M. (1972) *J. Am. Chem. Soc.* **94**, 5318.
21 Courtney, J. L. and Shannon, J. S. (1963) *Tetrahedron Letters* 13.
22 Budzikiewicz, H., Djerassi, C. and Williams, D. H. (1964) *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. II, p. 132. Holden-Day, San Francisco.
23 Betancor, C., Freire, R., Gonzalez, A. G. Salazar, J. A., Pascard, C. and Prange, T. (1980) *Phytochemistry* **19**, 1989.
24 Buckingham, J. (ed.) (1982) *Dictionary of Organic Compounds*, 5th edn, Vol. 1, p. 346. Chapman & Hall, London.
25 Nakanishi, K., Goto, T., Ito, S., Natori, S. and Nozoe, S. (1974) *Natural Products Chemistry*, Vol. 1, p. 365. Academic Press, London.
26 Ito, S., Kodama, M., Sunagawa, M., Oba, T. and Hikino, H. (1969) *Tetrahedron Letters* 2905.
27 Knight, S. A. (1974) *Org. Magn. Reson.* **6**, 603.
28 Rubinstein, I., Goad, L. J., Clague, A. D. H. and Mulheirn, L. J. (1976) *Phytochemistry* **15**, 195.
29 Holland, H. L., Diakow, P. R. P. and Taylor, G. J. (1978) *Can. J. Chem.* **56**, 3121.