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SOULATTRONE A, A C_{24} TERPENOID FROM *CALOPHYLLUM SOULATTRI**

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Key Word Index—*Calophyllum soulattri*; Guttiferae; bark; terpene; soulattrone A; 2D NMR; X-ray analysis.

Abstract—The structure of soulattrone A, a C_{24} terpenoid isolated from the bark of *Calophyllum soulattri*, was determined from its spectral properties and from X-ray crystallographic evidence. The complete assignment of the ^1H and ^{13}C spectra of this new skeleton was achieved.

INTRODUCTION

In our working program on the plants used by the tribal society of Archepelago of India, we found that calophyllolide [1], a 4-phenyl coumarin obtained from the seeds of *Calophyllum inophyllum* exhibited antiinflammatory activity [2, 3]. We studied then another plant of the same genus, *C. soulattri* [Mathur, K. S., personal communication], also known to have properties of reducing inflammation. *C. soulattri*, commonly named the Nicobar canoe tree, is a tall tree occurring in the forests of Tenasserim, Andaman and Nicobar Islands and Ceylon [4]. We had already analysed its seeds for lipid and protein content [5]. Gunasekara *et al.* [6] reported the isolation of soulattrolide, a phenyl-4 coumarin, from its bark in addition to taraxerone, taraxerol and β-sitosterol. We report now the structure elucidation of soulattrone A which we isolated from the bark of *C. soulattri*.

RESULTS AND DISCUSSION

Column chromatography of the benzene extract led to the isolation of a mixture of two closely related compounds from which the major one, soulattrone A (white crystals, mp 113–114°), was further separated by TLC.

The M_r of 388 and elementary composition $C_{24}H_{36}O_4$ of soulattrone A were obtained by high resolution mass spectrometry. The IR spectrum showed carbonyl absorptions at 1800, 1760, 1710 cm^{-1}, and a trisubstituted double bond absorption at 1670 cm^{-1}. The ^{13}C NMR proton noise decoupled spectrum accounted for all its 24 carbon atoms. Two carbonyl carbons from ketone groups, one from a lactone group, two quaternary carbons and two trisubstituted double bonds could be identified (Table 1). These spectroscopic data suggested the presence of a five membered lactone containing an additional ketone group and of side-chains containing double bonds and a ketone group. From the molecular formula, the remaining unsaturation revealed the presence of a second cycle.

The 500 MHz ^1H NMR spectrum showed signals due to eight methyl groups, four of which being linked to double bonds, and two to ethylenic protons. The couplings between the various protons were established by means of homonuclear two dimensional (2D) spectroscopy (^1H–^1H COSY). From inspection of the unambiguous cross peaks in the COSY spectrum and from the 2D heteronuclear ^{13}C–^1H correlated spectrum, we deduced directly three independent partial structures, **a**, **b** and **c** besides an additional $>C(Me)_2$ group.

The structural units **a**–**c** appeared to correspond to three lateral chains in soulattrone A. Several hypotheses might then be infered to elucidate the remaining bicyclic skeleton, but due to the lack of proton connectivities in this system its structure could not be determined without ambiguity only from the ^1H and ^{13}C NMR data. A single X-ray diffraction study was thus undertaken. The resulting structure **1** is depicted in Fig. 1 and the perspective view with the numbering scheme in Fig. 2.

The complete assignment of the ^1H and ^{13}C NMR spectra could then be accomplished (Table 1). The quaternary carbons were assigned on the basis of their ^{13}C NMR chemical shift values. The ethylenic methyl goups Me-11, Me-12, Me-23 and Me-24 were assigned taking into account the shielding of Me-12 and Me-24 arising from the *γ-gauche* interaction with the methylene groups C-8 and C-20, respectively [7]. The assignments of their ^1H NMR chemical shifts was then accomplished according to the results of the heteronuclear ^{13}C–^1H correlated spectrum.

The relative stereochemistry of soulattrone A (Fig. 2) determined by the X-ray data agreed with the values of the chemical shifts and proton–proton coupling constants. Me-18 (22.34 ppm) and Me-19 (16.15 ppm) were supposed to be in the equatorial and axial position, respectively, as deduced from their ^{13}C chemical shift values [8]. This was confirmed by a COSY long range coupling with incorporation of fixed delays in the pulse sequence, which opti-

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Table 1. ^1H (500.13 MHz) and ^{13}C (125.1 MHz) NMR spectra of soulattrone A ($CDCl_3$, δppm, TMS int. ref.).

C	H	δ^1H (multiplicity) J(Hz)	$\delta^{13}C$
1	—		172.22 *s*
2	—		56.89 *s*
3	—		205.11 *s*[a]
4	—		97.49 *s*
5	—		49.44 s
6	H_6	1.67 *m*	41.99 *d*
7	H_{7a}	1.525 *dd* $J_{7a-7b} = J_{7a-6} = 13.0$	41.13 *t*
	H_{7b}	2.134 *dd* $J_{7b-7a} = 13.0$ $J_{7b-6} = 4.6$	
8	H_{8a}	2.423 *dd* $J_{8a-8b} = 14.7$ $J_{8a-9} = 7.7$	26.24 *t*
	H_{8b}	2.373 *dd* $J_{8b-8a} = 14.7$ $J_{8b-9} = 7.4$	
9	H_9	5.050 *br dd*	117.10 *d*[c]
10	—		136.64 *s*[b]
11	Me_{11}	1.630 *br s*	25.69 *q*
12	Me_{12}	1.678 *br s*	17.98 *q*
13	—		201.84 *s*[a]
14	H_{14}	2.847 *ddq* $J_{14-15a} = J_{14-15b} = J_{14-17} = 6.9$	44.80 *d*
15	H_{15a}	1.308 *ddq* $J_{15a-14} = 6.9$ $J_{15a-15b} = 13.8$ $J_{15a-16} = 7.4$	24.63 *t*
	H_{15b}	1.746 *ddq* $J_{15b-14} = 6.9$ $J_{15b-15a} = 13.8$ $J_{15b-16} = 7.4$	
16	Me_{16}	0.866 *dd* $J_{16-15a} = J_{16-15b} = 7.4$	11.74 *q*
17	Me_{17}	0.847 *d* $J_{17-14} = 6.9$	15.87 *q*
18	Me_{18}	1.149 *s*	22.34 *q*
19	Me_{19}	1.089 *s*	16.15 *q*
20	H_{20a}	1.678 *m*	27.44 *t*
	H_{20b}	2.020 *m*	
21	H_{21}	4.990 *br dd*	121.80 *d*[c]
22	—		134.24 *s*[b]
23	Me_{23}	1.686 *br s*	25.75 *q*
24	Me_{24}	1.557 *br s*	17.81 *q*

[a, b, c] Attributions may be reversed.

mized detection of weak couplings [9]. The long range coupling between H-6 and Me-19 showed them to be in a *trans* diaxial position, the isoprenyl group at C-6 thus being in the equatorial position. Taking into account the axial position of H-6, the values of the ^3J coupling constants between the two protons H-7a, H-7b of the methylene group and the proton H-6 indicated H-7a and H-7b to be in the axial (J H7a-H6 = 13 Hz) and the equatorial (J H7b-H6 = 4.7 Hz) position, respectively. The absolute configuration has not yet been determined.

The structure of soulattrone A does not obey the terpene rules *sensu stricto*, but it might be considered as either a modified sesterterpene or a diprenyl sesquiterpene derivative.

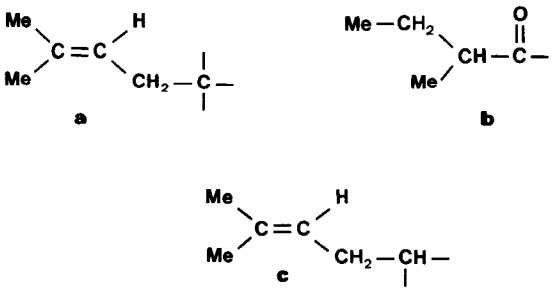

Fig. 1. Substructures in soulattrone A.

EXPERIMENTAL

The bark of *C. soulattri* Burm (syn *C. spectabile* Willd) was collected from the Andaman and Nicobar islands and a voucher specimen is deposited in the Herbarium Section of the National Botanical Research Institute (Lucknow).

Mps: uncorr. EIMS and high resolution measurements were obtained by direct inlet with 70 eV ionisation. ^1H and ^{13}C NMR spectra were obtained in the Fourier transform mode at 500.13 MHz for ^1H and 125.1 MHz for ^{13}C. Samples in $CDCl_3$ were referenced to int TMS. Two dimensional spectra (^1H–^1H COSY, ^1H–^1H COSY LR, ^{13}C–^1H COSY) were obtained by using the software DISN 85. X-ray data were measured using an automatic four-circle diffractometer.

Isolation of soulattrone A (**1**). Air-dried and ground bark of *C. soulattri* (4 kg) was extd with C_6H_6. After evapn of the solvent, the syrupy concentrate (135 g) was fractionated into hexane (5 × 500 ml) and Et_2O (4 × 500 ml) sol fractions. The hexane sol fraction (90 g) was subjected to CC over silica gel with petrol (60–80°) as eluent, furnishing first β-sitosterol (identified by comparison with an authentic sample) and then a crystalline mixture (mp 108–109°, EtOH) of soulattrone A and a minor related compound. Soulattrone A was further purified by TLC (silica gel Merck no. 5735, cyclohexane–CH_2Cl_2, 1:1); R_f = 0.4, mp 113–114° (MeOH–CH_2Cl_2). IR ν_{max}^{KBr} cm^{-1}: 2930, 2910, 2880, 1800, 1760 s, 1710, 1670, 1450, 1390, 1370, 1335, 1250, 1230, 1200,

Table 2. Fractional atomic coordinates ($\times 10^4$) for non hydrogen atoms of soulattrone A with e.s.d's in parentheses ($U_{eq} = 1/3 \sum_i \sum_j U_{ij} a_i^* a_j^*, a_i a_j$)

	X	Y	Z	U
0	844 (1)	2460 (3)	1651 (6)	57 (3)
0100	532 (1)	3916 (3)	593 (7)	67 (4)
0130	1538 (2)	702 (4)	4417 (11)	110 (6)
0300	1310 (1)	2923 (3)	6685 (6)	72 (4)
C-1	672 (2)	3395 (4)	2024 (10)	57 (6)
C-2	681 (2)	3605 (4)	4448 (9)	54 (5)
C-3	1044 (2)	2870 (4)	5207 (9)	57 (5)
C-4	977 (2)	1951 (4)	3673 (10)	57 (6)
C-5	545 (2)	1344 (4)	4501 (9)	57 (5)
C-6	117 (2)	2064 (4)	4421 (10)	60 (5)
C-7	214 (2)	3140 (4)	5328 (9)	59 (5)
C-8	753 (2)	4699 (3)	5102 (10)	64 (5)
C-9	1181 (2)	5136 (4)	4086 (11)	66 (6)
C-10	1575 (2)	5400 (5)	5080 (14)	87 (8)
C-11	1658 (3)	5293 (6)	7459 (15)	112 (10)
C-12	1974 (3)	5829 (7)	3824 (18)	151 (14)
C-13	1432 (2)	1399 (4)	3297 (11)	68 (6)
C-14	1764 (2)	1775 (4)	1541 (13)	90 (8)
C-15	2126 (3)	1024 (6)	975 (22)	166 (15)
C-16	2408 (3)	1367 (8)	−1059 (23)	208 (21)
C-17	1931 (3)	2791 (5)	1944 (22)	170 (17)
C-18	471 (2)	442 (4)	3003 (10)	73 (6)
C-19	635 (2)	972 (5)	6849 (9)	75 (7)
C-20	−332 (2)	1653 (4)	5528 (11)	75 (7)
C-21	−754 (2)	2243 (5)	4919 (12)	80 (7)
C-22	−1025 (3)	2803 (6)	6205 (14)	92 (9)
C-23	−945 (3)	2898 (6)	8563 (13)	122 (11)
C-24	−1443 (2)	3355 (7)	5286 (17)	141 (13)

1130, 1010, 1000, 910, 835. UV λ_{max}^{EtOH} nm (log ε): 206 (3.77), 220 (3.25), 297 (2.17). $[\alpha]_D^{22°}$ (EtOH): +157.3° (*c* 0.19 g/100 ml). MS *m/z* (rel. int. % molecular formula deduced from high resolution measurements): 388 (9, $[M]^+$, $C_{24}H_{36}O_4$; tr. 388.261, cal. 388.261), 320 (100, $C_{19}H_{28}O_4$), 252 (72, $C_{14}H_{20}O_4$), 196 (33, $C_{10}H_{12}O_4$), 123 (42, C_9H_{15}), 85 (82, C_5H_9O).

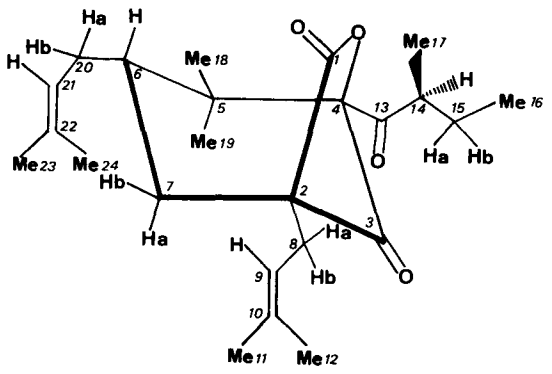

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Fig. 2. Relative stereochemistry of soulattrone A, 1.

X-ray analysis. A colourless prismatic crystal of 0.40 × 0.30 × 0.15 mm in dimensions was used. Crystal data: $C_{24}H_{36}O_4$, orthorombic, $a = 28.589$ (10), $b = 13.266$ (8), $c = 6.202$ (5) Å; $V = 2352.3$ Å^3, $Z = 4$, $D_c = 1.10$, space group $P2_12_12_1$, CuKα radiation. From 2488 reflexions, 1635 were significantly above background [$I > 3\sigma(I)$]. These reflexions were corrected for Lorentz and polarisation effects. No absorption correction was applied. The crystal structure was solved with direct methods programs [10]. The atomic coordinates and anisotropic thermal parameters were refined by least-squares methods [11] to discrepancy factors of $R = 6.34\%$ and $R_W = 6.60\%$, respectively. The function minimized in the refinement was $\Sigma_W(|F_o| - |F_c|)^2$ with a weighting scheme $w = [\sigma^2(F_o) + 0.002(F_o)^2]^{-1}$. The H atoms were introduced to their theoretical position (C-H = 1.08 Å) and assigned the equivalent isotropic thermal factor of the bounded C atom. The extremity of the three chains branched on the heterocycle are specially affected by thermal motion. No peaks above 0.3 e/Å^3 are observed on the final difference synthesis. The anisotropic thermal parameters, bond distances and bond angles for this work are available on request to the Director of the Cambridge Crystallographic Data Centre University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. The list of observed and calculated structure factors is available from the authors at the Institut de Chimie des Substances Naturelles.

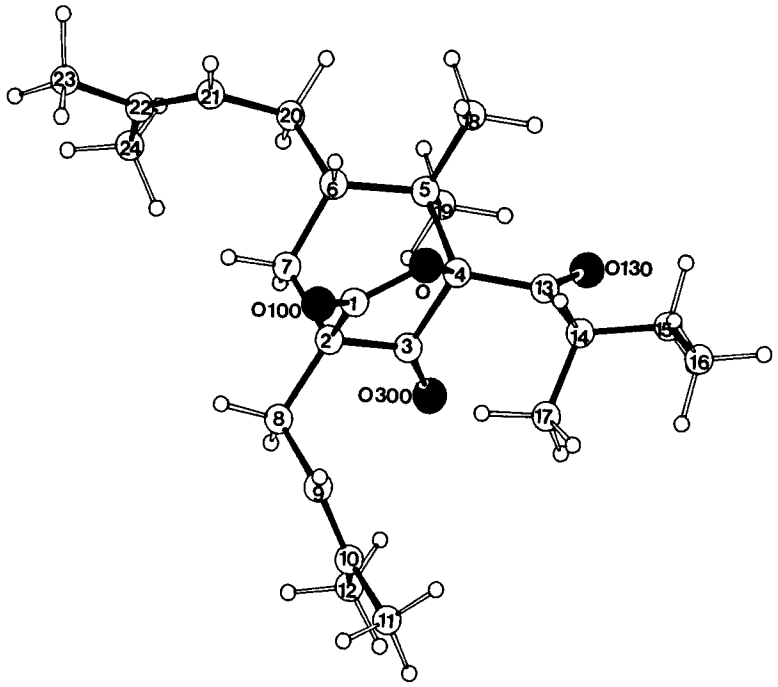

Fig. 3. Perspective view of soulattrone A showing the crystallographic numbering scheme.

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