The Structure of Wightianone, the Pigment of a Clathrate from *Calophyllum wightianum*

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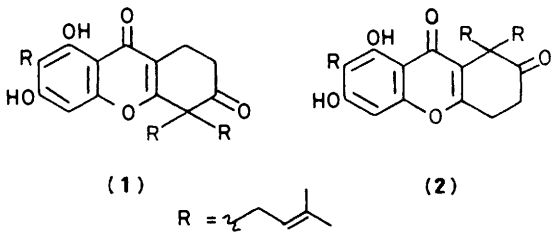
From the heartwood of *Calophyllum wightianum* T. Anders has been isolated, in small quantities, a clathrate composed of four molecules of a pigment, wightianone, to one of palmitic acid. Other fatty acids are present in small amounts. Wightianone is shown to be identical with zeyloxanthonone and to have the structure (**1**) {7,9-dihydroxy-4,4,8-tris(3-methylbut-2-enyl)-1,2-dihydroxanthene-3-dione}. The isomeric structure (**2**) for the pigment was rejected on the basis of the aromatic solvent-induced shifts and because of biosynthetic considerations, *i.e.*, the occurrence of *gem*-dialkylation in resorcinol and phloroglucinol but not in quinol nuclei.

Calophyllum wightianum T. Anders (Guttiferae) is a tree of moderate size occurring in the evergreen forests of the western Ghats.[1] Even in its natural habitat it is sparsely distributed and, perhaps owing to this, only cursory work has been done on it,[2] whereas *Calophyllum inophyllum* has been investigated both in India and abroad.[3,4]

During a study of the extractives of this plant a pigment was isolated that proved to be a clathrate, formed from palmitic acid and a xanthone derivative nemed wightianone. In a preliminary communication,[5] wightianone was assigned structure (**1**) which, except for the orientation in the hydroaromatic part, is the same as the structure (**2**) earlier proposed by Karunanayke *et al.*[6] for zeyloxanthonone isolated from *Calophyllum zeylanicum*. Later, direct comparison revealed the two pigments to be identical. Since we are in agreement on other matters, we report here in full detail only our study of the clathrate and our reasons for rejecting the structure (**2**) which other workers continue to regard as correct.[7]

Extraction of the heart-wood of *Calophyllum wightianum*, collected from Goa yielded a light yellow solid of which, after extensive chromatographic purification and crystallisation, about 500 mg was obtained. It was sharp-melting, gave a single spot on chromatostrips, and appeared to be pure. The accurate mass of the molecular ion, *m/z* 450.2493, supplied the molecular formula $C_{28}H_{34}O_5$, but this was found to be somewhat at variance with the C, H ratio indicated by microanalysis and, further, did not accord with the ^{1}H n.m.r. spectrum which showed a total of 40 protons. The discrepancy was eventually traced to impurities (fatty acids) that are not eliminated through application of the usual methods of purification and are not readily observable by u.v., i.r., or mass spectroscopy or t.l.c. methods for various reasons. Even when the pigment was converted into its acetate or methyl ether the impurity remained, and the lack of correspondence between the ^{1}H n.m.r. and mass spectra persisted, the methylated (CH_2N_2) material showing a small band at 1 750 cm^{-1} in the i.r. spectrum consistent with the presence of a trace of a carboxylic ester. The ^{1}H n.m.r. spectrum does not show any fractional protons and is so well resolved as to be mistaken for that of a pure compound. Later, a 6 H 'singlet' at the highest field was assigned to the methylenic protons of a fatty acid impurity; a triplet, barely perceptible above the noise, was ascribed to the terminal methyl group (δ 1.08) and another to the methylene group attached to the carboxy group (δ 2.38) (Figure). Formed by catalytic hydrogenation, the hexahydro derivative of wightianone crystallised without inclusions, thus confirming these interpretations. Only the roughest integration of the weak triplets



was possible, but it indicated the size of the acid to be between dodecanoic and stearic, consonant with the reported isolation of glycerides of palmitic, stearic, and oleic acids from seed kernels of the plant.[2] The failure of crystallisation techniques to rid the compound of the impurity, or more significantly, reduce its content, suggested that it might be present in a specific molecular ratio, and that the compound might be a clathrate of the fatty acid with the pigment. Confirmation of this was obtained through crystallisation of the clathrate from methanol saturated with urea so that the urea inclusion complex of the fatty acid crystallised out preferentially leaving the solution enriched in the pigment. Repeated crystallisation of this kind cleared the pigment of most of the impurity.

After a final crystallisation the ^{1}H n.m.r. spectrum was compared with that obtained before treatment with urea and showed a very substantial reduction in the height of the intrusive signal at δ 8.80 (Figure). The m.p. of the purified material was about 35 °C *lower* than that of the original, a very strong indication that an inclusion complex was in hand. In order to determine its nature more exactly, the fatty acid fraction was recovered from the urea crystallisate and, after methylation with diazomethane, was subjected to g.l.c. which showed that palmitic acid constituted the largest component besides a little myristic acid and stearic acid. Relative proton intensities in the ^{1}H n.m.r. spectrum showed pigment and fatty acid to be in the molar ratio of *ca.* 4:1, as are many similar inclusion compounds between deoxycholeic acid and fatty acids.[8]

Finally, the clathrate was reconstituted by crystallising the pigment from methanol solution to which authentic palmitic acid had been added. The H n.m.r. spectrum of the material resulting from this crystallisation not only reverted to the original pattern, but even showed a slight enhancement in the height of the intrusive signal, since clathrates are seldom perfectly stoicheiometric (Figure). A sample of zeyloxanthonone kindly provided by Dr S. Sotheeswaran crystallised with palmitic acid in the same way.

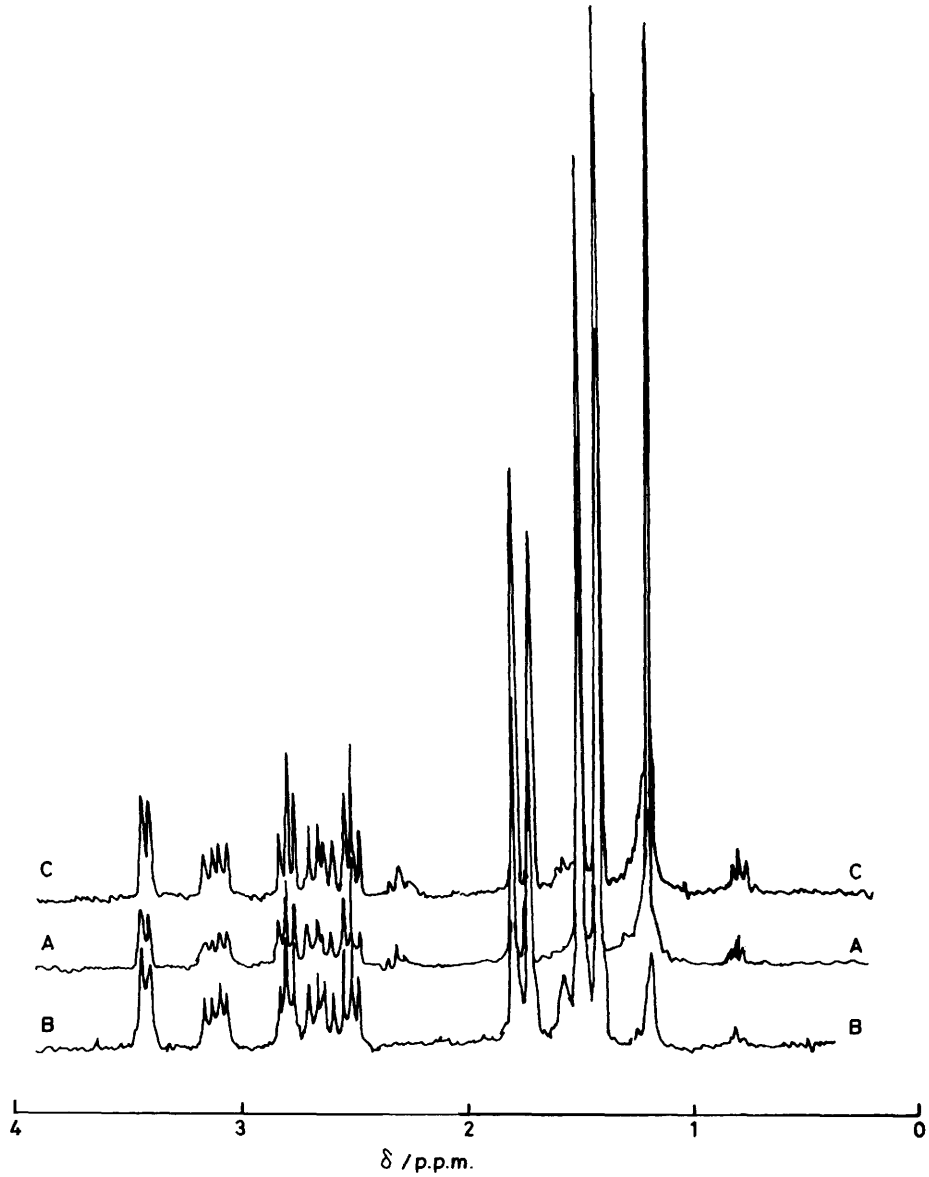


Figure. ^{1}H N.m.r. spectra of wightianone (**1**) determined at 220 MHz in $CDCl_3$: A, pigment from the plant; B, pigment after urea treatment; C, pigment as in B, but crystallised in the presence of palmitic acid

With the molecular formula firmly established and the nature of the intrusive signal in the ^{1}H n.m.r. spectrum settled, the structure of the pigment could readily be solved by conventional methods already outlined by us[5] and others.[6,7] Only the disputed problem of orientation requires additional discussion.

Our analysis of the ^{1}H n.m.r. spectrum (Table) of the pigment led to the view that the methylenic parts of the *gem*-diprenyl grouping appear as two coincident ABX systems because the methylene protons are diastereoisotropic.[5] Sultanbawa and his colleagues[7] clearly accept our analysis while arguing that structure (**1**) fails to account for the fact that the lateral components of the methylene groups resonate at substantially different fields ($\Delta\delta$ *ca.* 0.3). They suggest that there would be little difference, and in support they quote a reported spectrum[9] for compound (**3**) in which the two *gem*-methylene groups are assigned only one band; *i.e.*, all four protons appear to have the same chemical shift. Structure (**3**) is hardly a satisfactory model, however, because the prenyl groups are symmetrically situated with respect to a series of magnetically anisotropic groups. Moreover, this compound is presumably tautomeric and in the tautomer the substituents flanking the *gem*-diprenyl group become almost exactly equivalent. In the absence of valid models we can see no way of deciding whether the magnetic environment of the prenyl groups would be more (or less) symmetrical in structure (**1**) than in (**2**) and reject this line of argument.

In basic media tetrahydroxanthone (**4**) undergoes a slow, but selective, exchange with deuterium oxide at position 4, which is activated by the pyrone carbonyl group.* A similar experiment with wightianone was attempted because in structure (**1**) only two protons should be replaceable, whereas in structure (**2**) all four methylenic protons should be. Unfortunately, wightianone was too unstable in base for the method to be applied.

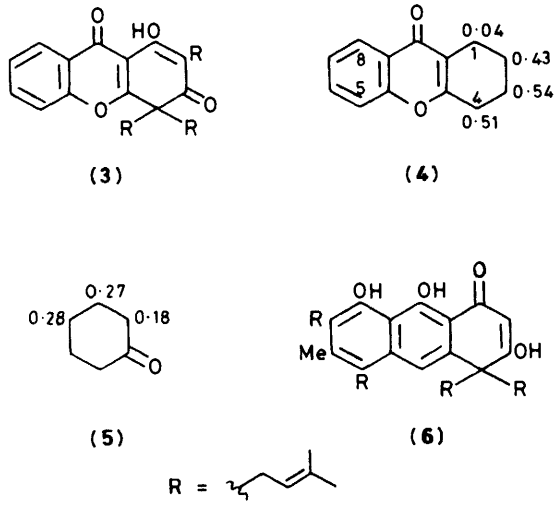
The problem was then studied through measurement of benzene-induced shifts[10] in tetrahydroxanthone and cyclohexanone and computing from these the expected values for

* We thank Mr. D. J. Chard for conducting these experiments.

Table. ^{1}H N.m.r. spectra at 220 MHz and solvent shift (ASIS) results*

	Wightianone (**1**)†			Model chromone (**16**)				
Proton	δ $CDCl_3$	δ C_6D_6	Δ $CDCl_3$–C_6D_6	δ $CDCl_3$	δ C_6D_6	Δ $CDCl_3$–C_6D_6	Multiplicity††	*J*(Hz)
ArH	6.34	6.11	0.23	6.34	6.25	0.09	s	
Chromone 3-H				5.98	5.69	0.29	q	1
OMe				3.87	3.30	0.57	s	
Chromone 2-Me				2.36	1.68	0.68	d	1
Chromone 5-OH	13.42	14.02	−0.60	12.74	13.42	−0.68	s	
$CH_2CH_2C{=}O$	2.86	2.27br	0.59				t	7.7
$CH_2CH_2C{=}O$	2.56	2.27br	0.29				t	7.7
gem-Prenyl								
$=CH_X$ (2H)	4.82	5.03	−0.21				t	8, *ca.* 7
CH_AH_B (2H)	3.16	3.45?	−0.29?				dd	14, *ca.* 7
CH_AH_B (2H)	2.70	3.04	−0.34				dd	14, 8
$=CMe_2$ (6H)	1.56	1.54	0.02				s	
$=CMe_2$ (6H)	1.48	1.47	0.01				s	
Aryl prenyl								
=CH	5.30	5.32	−0.02	5.17	5.35	−0.18	t	7.7
CH_2	3.48	3.54	−0.06	3.36	3.44	−0.08	d	7.7
$=CMe_2$ (3H)	1.86	1.63	0.23	1.81	1.76	0.05	s	
(3H)	1.79	1.54	0.15	1.68	1.66	0.02	s	
$(CH_2)_n$	1.26	1.35					br s	

* Me_4Si as internal standard. Relative intensities are those required by assignments and are quoted only when necessary to prevent confusion. Spin couplings were confirmed by double irradiation experiments not recorded here. † Zeyloxanthonone gave identical results except for the bands assigned to $(CH_2)_n$ which were absent. †† For $CDCl_3$ solutions only.

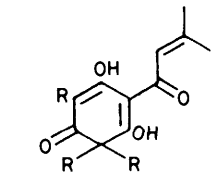


structures (**1**) and (**2**). The relevant solvent shifts for tetrahydroxanthone (**4**) and cyclohexanone (**5**) are shown in these diagrams, the calculated solvent shifts for structure (**1**) being 0.61 and 0.31 and for (**2**) being 0.72 and 0.78. The observed values, 0.59 and 0.29, are almost exactly those required by structure (**1**) (Table). Zeyloxanthonone shows precisely the same solvent shifts. Thus, on these grounds both pigments must be assigned structure (**1**). This is very surprising, because the common oxygenation pattern in plant xanthones is not that in structure (**1**) but that in (**2**).

Though persuasive, the solvent shift method cannot be regarded as absolute. It assumes that solvent shifts for methylene groups will be roughly additive, a property extensively tested only for methyl groups so far.[10,11] It also assumes that steric (bulk) effects exerted by the *gem*-prenyl groups will not interfere, a matter that has not been tested in any sufficiently close model. However, there is a biosynthetic or chemotaxonomic argument that also points to structure (**1**) rather than to the isomer.

The question of xanthone biosynthesis has been well summarised in extensive discussions of xanthones of the Guttiferae and Gentianaceae.[12] Rezende and Gottlieb[13] believe, 1,3,5,6- and 1,3,6,7-tetraoxygenation patterns to be the primitive ones in xanthones, others being derived by modification. Obviously, removal of a 5- or 7-oxygen atom, as appropriate, will leave a 1,3,6-trioxygenated nucleus as required for structure (**1**), though usually it is the 6-oxygen that is lost leaving either the 1,3,5-pattern or the 1,3,7-pattern needed for structure (**2**). It is interesting that the anthrone harunganin[14] (**6**), also found in the Gutteriferae, has the oxygenation pattern we suggest for structure (**1**). Whether xanthones and anthrones are kindred in the higher plants is not known, but they are in the lower plants.[15]

Thus, the common oxygenation pattern taken alone favours structure (**2**) without actually eliminating (**1**). Another feature, however, seems to us to reverse this preference. As is well established, methyl and prenyl groups are inserted into preformed phenolic nuclei or their immediate precursors.[15] Now so far as we are aware, biosynthetic *gem*-dialkylation is found only when 1,3-diketonic systems are involved, *i.e.*, only in resorcinol and phloroglucinol derivatives. This observation is paralleled *in vitro*, since methylation and prenylation affords *gem*-dialkyl compounds only when 1,3-diones or their enolic counterparts, resorcinol and phloroglucinol rings, are the substrates.[9,16,17] The model compound (**3**) was obtained in this way.[9] Relevant examples of natural products are lupulone (**7**) and its congenors,[17] the fern bisphenols including desaspidin[18] (**8**), myrtocommulone A[19] (**9**), compounds related to xanthochymol[20] (**10**), hyperforin,[21] members of the inophylloidic acid series,[22] dalrubone and methoxydalrubone (**11**),[23] and of course, harunganin[14] (**6**), along with several related compounds described recently.[24]



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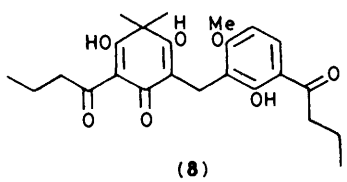

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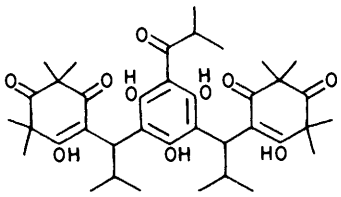

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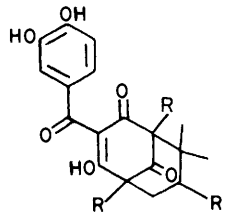

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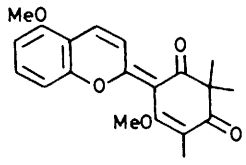

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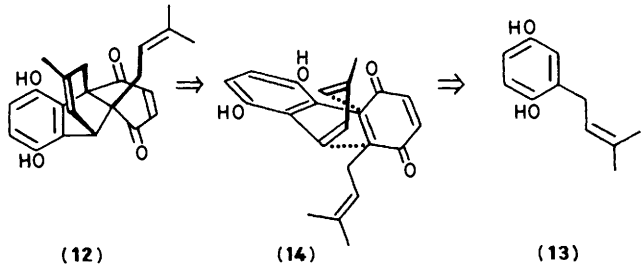
We found one case in which a quinol nucleus might have been *gem*-dialkylated, but here another origin is much more convincing. The compound is microphyllone[25] (**12**). Instead of diprenylation of a diphenyl derivative, this could be produced by *mono*-prenylation of quinol followed by oxidative coupling of the product (**13**) *etc.*, as in the Scheme. The quinhydrone (**14**)

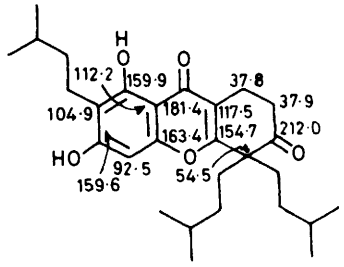


(12) (14) (13)

Scheme.

so formed would then be highly susceptible to internal 1,4-cycloaddition (dotted lines) leading at once to the product (Scheme). Hence, we do not regard microphyllone as abrogating the general rule, and take these observations as very strong independent evidence for structure (**1**).

It has been previously suggested that the presence of jacareubin and/or its putative precursor may be of taxonomic value in identifying *Calophyllum* species.[26] Only in the Indian variety of *Calophyllum inophyllum* are these metabolites absent.[27] Our results also demonstrate the unique nature of the Indian varieties of *Calophyllum* since apart from the xanthone only β-sitosterol and β-amyrin were isolated, there being no indication of the presence of jacareubin or its precursor.



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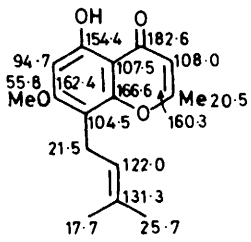

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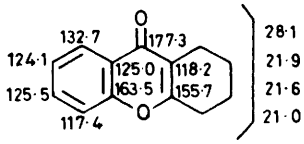

(17)

Experimental

M.p.s were determined with a hot-stage microscope and are uncorrected. Light petroleum refers to the fraction with b.p. 60—80 °C. Without other specification, n.m.r. spectra were determined in $CDCl_3$ with Me_4Si as internal standard. Only the major bands are quoted for i.r. spectra.

Isolation of Wightianone–Fatty Acid Clathrate.—Air-dried heartwood of *Calophyllum wightianum* (5 kg) was chopped into small pieces and extracted twice with hot benzene. The extract was reduced to a small volume under reduced pressure and the viscous residue (50 g) was adsorbed on silica gel (100 g) and chromatographed. Elution with light petroleum – benzene after the removal of oily material with light petroleum yielded β-sitosterol (1.2 g), β-amyrin (0.7 g), and crude pigment which was purified by repeated chromatography and crystallisation from benzene–light petroleum to yield bright yellow needles of *wightianone–fatty acid clathrate* (500 mg), m.p. 168 °C (Found: C, 74.0; H, 8.05. Calc. for $C_{28}H_{34}O_5$: C, 74.62; H, 7.61%. Calc. for 3 $C_{28}H_{34}O_5 \cdot C_{16}H_{32}O_2$: C, 74.66; H, 8.40%. Calc. for 4 $C_{28}H_{34}O_5 \cdot C_{16}H_{32}O_2$: C, 74.67; H, 8.23%. Calc. for 5 $C_{28}H_{34}O_5 \cdot C_{16}H_{32}O_2$: C, 74.65; H, 8.12%); $\lambda_{max.}$ (MeOH) (log ε)* 324sh (3.50), 298 (3.92), 258 (4.14), 254sh (4.13), and 232 nm (4.14); $\lambda_{max.}$ (MeOH–NaOH) 341 (4.12), 269 (4.22), and 228 nm (4.43). [For peucenin under the same conditions: $\lambda_{max.}$ (MeOH) 332sh (3.50), 298 (3.92), 256 (4.15), 251 (4.16), and 232 nm (4.17); $\lambda_{max.}$ (MeOH–NaOH) 342 (3.99), 270 (4.16), 265 (4.14), and 231 nm (4.39)], $\nu_{max.}$ (Nujol) 3 118, 1 700, 1 645, 1 590, 1 190, and 1 080 cm^{-1} *m/z* (E.I.) (rel. intensity) 450.2493 (Calc. for $C_{28}H_{34}O_5$: 450.2406) (40), 396 (65), 394 (70), and 381 (100) ($M^+ - C_5H_9$). The ^1H n.m.r. spectrum is detailed in the Table.

Resolution of the Clathrate by Urea.—To the clathrate (60 mg) in hot methanol (20 ml) was added urea to saturation and the solution was allowed to cool. The crystals were removed, the filtrate concentrated, and crystallisation again permitted. The mother-liquor was mixed with benzene (10 ml) and washed with water (3 × 5 ml), dried (Na_2SO_4), and kept under reduced pressure to remove volatile materials. The residue showed in the ^1H n.m.r. spectrum a band of reduced intensity at δ 1.26. The treatment was repeated giving a product with only a weak band

* Disregarding the fatty acid components.

at this point (Figure), which crystallised from benzene as yellow prisms (*ca.* 18 mg), m.p. 128—133 °C (zeyloxanthonone[6] has m.p. 137 °C).

The pigment (11 mg), m.p. 128—133 °C, and palmitic acid (1.0 mg; molar ratio 6:1) were mixed and crystallised from benzene to reconstitute the clathrate which formed massive hexagonal prisms, m.p. 156—158 °C, with a ^{1}H n.m.r. spectrum almost identical with that of the original clathrate (Figure). A sample of zeyloxanthonone gave an exactly similar clathrate with palmitic acid.

All urea-containing fractions from the resolution were dissolved in the minimum amount of water and extracted with CH_2Cl_2 (2 × 10 ml) and ethyl acetate (2 × 10 ml). The combined extracts were washed with water (2 × 2 ml), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was treated for five minutes with ethereal diazomethane, the solvents removed under reduced pressure, and the product analysed by gas chromatography [2M column packed with PEGS (20%) on Celite and run at 185 °C]. The main band coincided with methyl palmitate (100) with subsidiary bands corresponding to methyl stearate (3.2), and methyl myristate (2.9) (relative intensities in parentheses).

Hydrogenation.—The original clathrate (50 mg) in methanol (15 ml) was shaken under hydrogen at 20 °C with Pd/C (10%; 20 mg) for 6 h. After the usual work-up, the product in benzene was passed through a small column of silica and then crystallised from acetone–light petroleum on methanol to give hexahydrowightianone (**14**) as cream-coloured needles (30 mg), m.p. 158 °C. $\nu_{max.}$ (Nujol) 3 270, 1 700, 1 650, 1 620, and 1 580 cm^{-1}. *m/z* (C.I.) 457; *m/z* (E.I.) 456 (Calc. for $C_{28}H_{40}O_5$: *m/z* 456). ^{13}C N.m.r. assignments for the nuclear atoms are given in structure (**14**), along with the values for the related compounds, peucenin methyl ether (**15**) and 1,2,3,4-tetrahydroxanthone (**16**).

Acknowledgements

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