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THE STRUCTURE DETERMINATION OF TWO NEW ACYLPHLOROGLUCINOLS FROM *MYRTUS COMMUNIS* L.

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Abstract—Structures for two new acylpholorglucinols, myrtucommulone A (1) and myrtucommulone B (2) have been deduced from a detailed study of the NMR, IR and mass spectra of these substances, and their simple derivatives and degradation products. The compounds contain phloroglucinol and syncarpic acid (1,1,3,3, tetramethyl-cyclohexa-2,4,6-trione) residues, and are closely related chemically to fern constituents such as filixic acid, as well as to kosins and uliginosins isolated from higher plants. Compounds 1 and 2 are unique in having an isobutyl bridge connecting the rings. Compound 1 exhibits strong antibacterial activity against gram positive bacteria.

Myrtus communis L. is a shrubby plant belonging to the family Myrataceae sub family Myrtoideae, and is widely distributed in the Mediterranean area. An investigation of the chemical constituents of the plant was undertaken as part of a general search for natural products with potentially useful biological activity. From the extract of the plant for which the occurrence of antibacterial properties was reported,[1] we isolated a pale yellow crystalline compound which was responsible for the biological activity. Herewith we report the structure elucidation of the latter and a closely related compound which were called myrtucommulone—A (1) and myrtucommulone—B (2).

Myrtucommulone—A, m.p. 185° is highly antibacterial to gram positive bacteria (1γ/ml). Elemental analysis of 1, together with the high resolution mass spectrum *vide infra* were consistent with $C_{38}H_{52}O_{10}$. Furthermore, the mass spectrum revealed, in addition to the parent ion, an impurity present to the extent of *ca* 15% and having a molecular weight of 682 [$C_{39}H_{54}O_{10}$]. Efforts to remove this impurity by repetitive chromatography on different columns, as well as recrystallization from several solvents, were to no avail.

The IR spectrum of 1 showed broad absorption in the 2200–3500 cm^{-1} region and this, coupled with intense peaks at 1580 and 1620 cm^{-1} suggested the presence of an enolic 1,3-diketo system or a 2-hydroxyaryl ketone.[2-4] Another saturated ketone is indicated by the absorption at 1710 cm^{-1}. Further evidence for the former moiety was obtained from a positive ferric chloride test.

The NMR spectrum (Fig 1) of 1 turned out to be quite complicated, strongly pH dependent, and changed in various $CDCl_3$† solutions. Nevertheless it proved most informative since it contained several features that reveal a number of important structural details. First and foremost three well defined groups of signals attributed to methyls could be observed at the high-field region δ 0·82 (12H), δ 1·22 (6H), and δ 1·30–1·50 (24H) accounting for 42 (14-Me groups) out of the 52 protons of the molecule. Among three additional multiplets appearing at δ 3·06 (2H), δ 3·82 (2H) and δ 4·16 septet (1H) the first, as could be seen from a double irradiation experiment, was coupled with the superimposing methyl signals at δ 0·85. The latter septet corresponds to the proton vicinal to the methyls giving rise to the signal at δ 1·22; this septet, sharp in $CDCl_3$ and broadened in CCl_4, occurs in a region typical for $(CH_3)_2\underline{C}HCO$— although somewhat further deshielded. The existence of this iPrCO group was further supported by the easy loss of 43 m.u. in the mass spectrum of 1 *vide infra.*

The duplicity of both the latter and the above mentioned methyls was found to be both solvent, as well as temperature dependent, pointing to a possibility of several isomeric forms.

Perhaps the most interesting aspect of the NMR spectrum of 1 was the presence of at least twenty-five signals at δ 10·20–11·75 (3H), δ 13·15–13·35

†Most of the commercial $CDCl_3$ tested, was found to be acidic after short exposure to air.

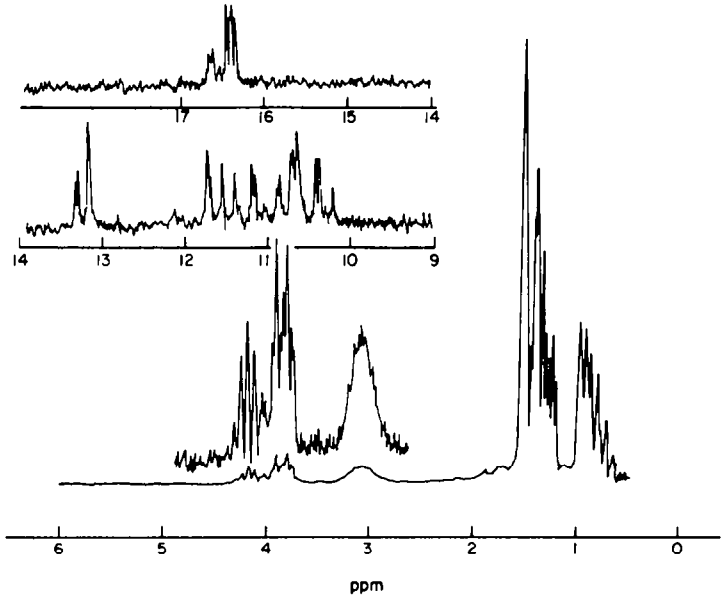


Fig 1. NMR spectrum of compound 1.

(1H), and δ 16·30–16·70 (1H)—all due to hydroxylic protons disappearing upon briefly shaking the sample with D_2O. The large number of signals was consistent with the above mentioned Me duplicity (at δ 0·85 and 1·22) pointing to a mixture of at least 5 isomers. The peaks at 16·30–16·70 ppm are at a remarkably low position but as such proved very informative since the suspected β-triketone site constitutes one of the few system known which absorb in this region.[3,4]

The addition of a trace of ammonia gas[5] to the NMR tube caused an immediate dramatic coalescence of the three MeI groups (Fig 2), namely the complex at δ 0·85 changed into two broad doublets at δ 0·72 (J = 6·5 Hz) and 0·83 (J = 6·5 Hz), the signal at δ 1·22 changed into a doublet at δ 1·12 (J = 7 Hz) and all other methyls gave rise to a quite sharp singlet at δ 1·30 ($\Delta W_{1/2} = 4·5$ Hz). Furthermore the multiplet at 3·06 sharpened into a septet, which by additional irradiation at δ 0·80 changed into a singlet, thus confirming the existence of two isopropyl groups in additoion to the iPrCO group. A similar sharpening could also be seen on the multiplet at 3·82. The enolic proton signals, appeared as a rather broadened signal between 4·5 to 8 ppm, moving steadily to higher field on continued addition of

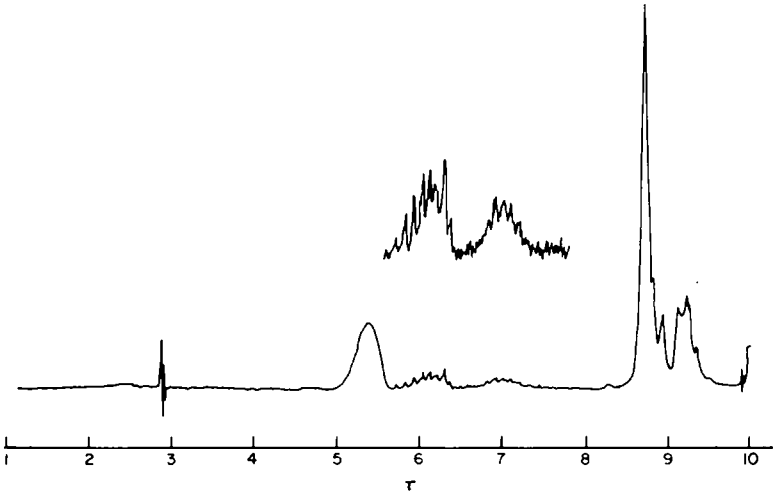
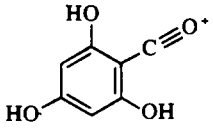


Fig 2. NMR spectrum of compound 1 + NH_3-gas.

small amounts of the base. The above described behaviour which is well known for filixic acid,[2] tasmanone and other β-triketones[5] unequivocally indicated a similar tautomer mixture in the case of **1**, where at least eight reasonable tautomers could be described.

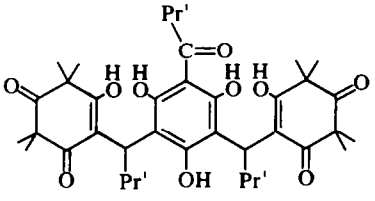
Further information was obtained from the analysis of the mass spectrum of **1**: among other peaks the one appearing at 153,0200 ($C_7H_5O_4$) could be easily attributed to ion **a** (or one out of the other possible isomers).



Ion **a**

As no aromatic protons were evident in the NMR spectrum of **1**, a 1,3,5-trisubstituted phloroglucinol derivative was suggested, a structure which may resemble the fern phloroglucides i.e. the various filixic acid derivatives.[2] In the knowledge of the phloroglucinol derivatives sensitivity towards base, the alkaline cleavage of **1** was performed, leading to 1,1,3,3-tetramethyl phloroglucinol (syncarpic acid)[6]—two equivalents, phloroglucinol and traces of a substituted phloroglucinol which was not identified.

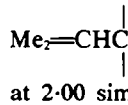
Based on the degradation products as well as the NMR and IR spectra the following structure is suggested for **1**:



1

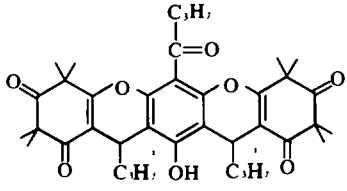
The mass spectrum fragmentation pattern of **1** (Fig 3) shows great similarity to that of the acyl phloroglucinols[7] (Scheme 1) and is in full agreement with the proposed structure of **1**.

Attempts to acetylate or methylate **1** did not lead to clean products; on the other hand its acidic treatment, namely boiling in benzene in the presence of *p*-TsOH, or in alcohol in the presence of dil-HCl, gave rise to a new compound-3. Compound **3**, $C_{38}H_{48}O_8$ M.Wt 632, m.p. 290° (dec); ν_{max}^{KBr} 3350 (OH), 2950, 2910, 1710 (CO), 1650, 1600, 1170, 980 cm^{-1}; exhibited a much simpler NMR spectrum then **1**: δ 0·80 d(J = 6·5 Hz; 12H), 1·22 d and 1·24 d(J = 6·5 Hz; 6H), 1·45 brs (18H), 1·54s (6H), 2·00m (2H), 3·12 septet (J = 6·5 Hz, 1H), 4·63 d (J = 3·5 Hz; 2H) and 8·40 brs (1H, disappearing when adding D_2O). Double irradiation experiments clearly showed the existence of the following moieties; **a**. iPrCO group (irradiation at 3·12 changed the two doublets at δ 1·22 and 1·24 to a broad singlet). **b**. two



groups, (irradiation of the multiplet at 2·00 simultaneously changed the doublets at δ 0·80 (12H) and the one at 4·63 (2H) into singlets; the reverse irradiations gave the expected results). The latter two isobutyl groups suggested in the parent compound **1** are thus unequivocally confirmed. The mass spectrum of **3** differs completely from that of **1**. Only one strong peak could be observed at *m/e* 589·2789 ($C_{35}H_{11}O_8$, $[M\text{-iPr}]^+$ again accompanied by the higher homologue 603·2957 ($C_{36}H_{43}O_8$) *ca* 13%). The parent peak at *m/e* 632 could be seen only in very low intensity (~ 0·1%).

The fragments which appeared in the mass spectrum of **1** entirely were absent in that of **3**. The formation of **3** from **1** can be easily explained by the acid catalysed hemi-ketal formation between the phenolic hydroxyl and the neighbouring ketone, followed by elimination to give the stable benzopyran system. This leads in the mass spectrum to the formation of the stable ion $(M\text{-iPr})^+$. Based on the above data, the following structure is suggested for **3**.



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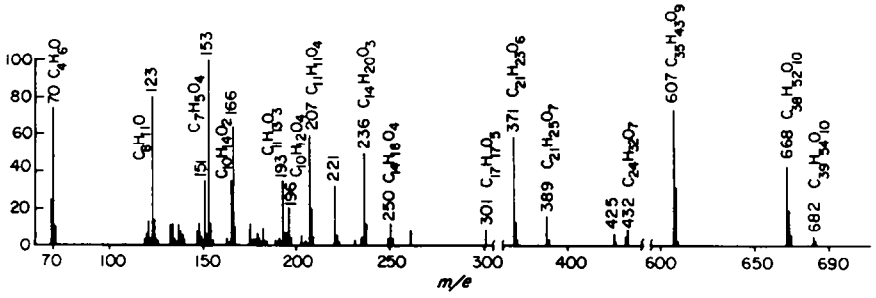
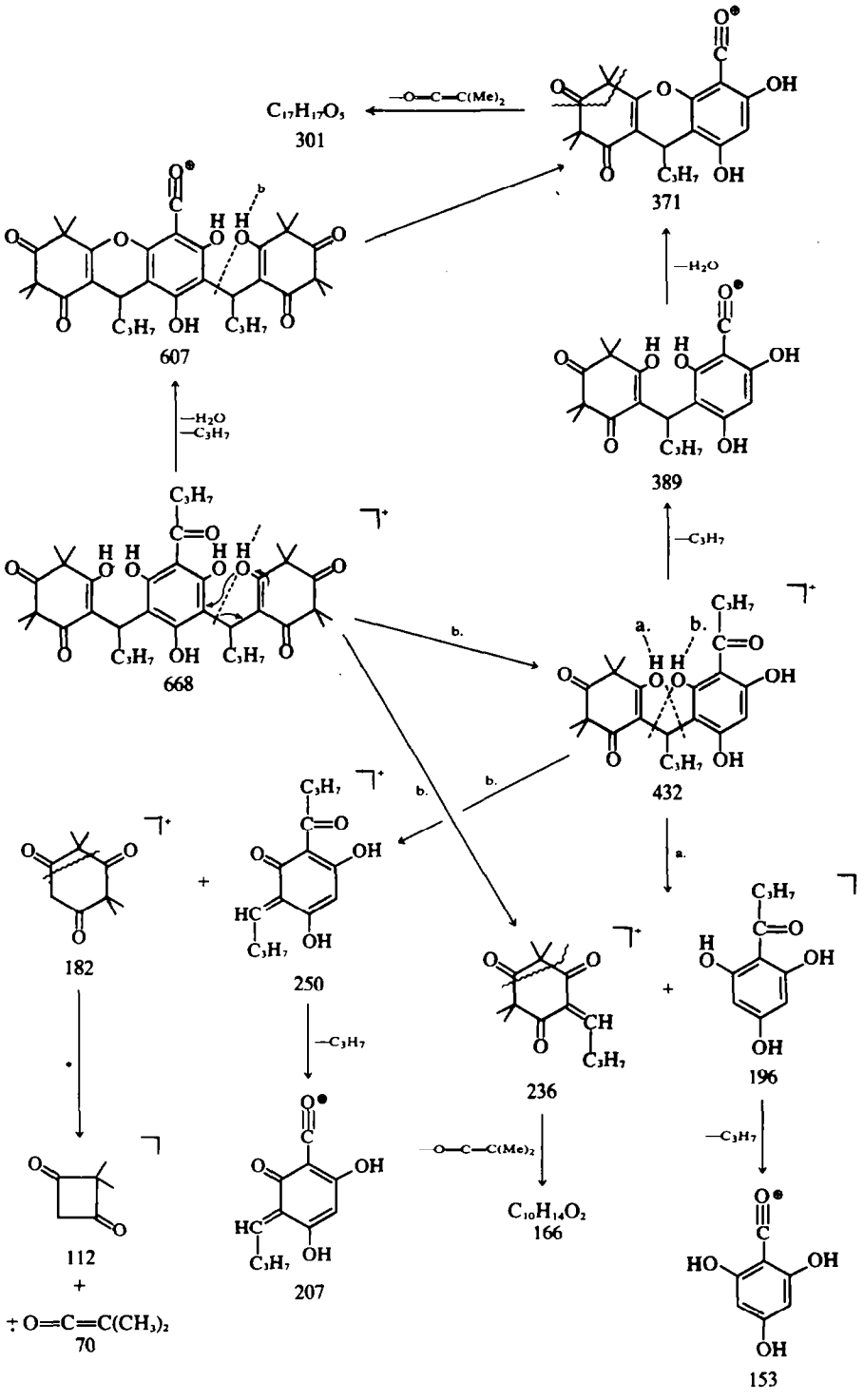


Fig 3. Mass spectrum of compound **1**.



SCHEME 1. Mass spectra fragmentation pattern of compound 1.

*Similar fragmentation was observed for syncarpic acid.

Turning now to myrtucommulone—B(2), assuming similar biosynthetic precursors, the structure elucidation was straightforward. Compound 2, $C_{24}H_{30}O_6$, ν_{max}^{KBr} 3300 (OH), 2930, 1715 (CO), 1650, 1600, 1150, 1035, 990, 840 cm^{-1} showed the following moieties in the NMR spectrum: (Fig 4).

(a) $Me_2{=}CH\overset{|}{C}H$ group; δ 0·80 d and 0·82 d (J = 6·5 Hz, 6H), 1·94 m (1H) and 4·31 d (J = 3 Hz; 1H), the latter doublet resembling the parallel isobutyl proton in 3. The location of the protons relative to each other in this site was estimated by a spin decoupling experiment. (b) iPrCO group: δ 1·25 d (J = 6·5 Hz; 6H) and δ 3·92 septet (J = 6·5 Hz; 1H). (c) four Me groups, δ 1·38s (3H), δ 1·43s (6H), and δ 1·62s (3H)—in a similar position to the syncarpic acid site methyls in 1. (d) one aromatic proton, at δ 6·26s, between two hydroxylic groups, in a similar position to the corresponding one in pseudo-aspidinol[2] (6·19s), δ 0·95 br (1-OH) and 14·30s (1-OH).

Alkaline treatment of 2 resulted in one equivalent of syncarpic acid and phloroglucinol per one equivalent of 2. The overall picture of the mass spectrum of 2 was similar to that of 3—no base peak and strong $[M\text{-}iPr]^+$ (m/e 371) fragment accompanied by $[M\text{-}iPr\text{-}70]^+$ (m/e 301).

Based on the above data the following structure is suggested for 2.

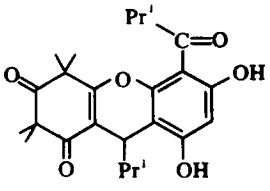
Addition of NH_3-gas to the NMR tube of 2 only caused the collapsing of the two doubtlets at δ 0·80 and 0·82 to one at 0·80. This may be due to the presence, in the tautomerization equilibrium, of a methine quinone tautomer in which the asymmetric center is cancelled. Compound 2 in contrast to 1 could be methylated to 4, the dimethyl derivative, but remained unchanged under acidic treatment as expected.

Myrtucommulone A and B are chemically related to the acylphloroglucinols of the Dryopteris species[8] and their occurrence in a higher plant is of interest.

Myrtucommulon is similar to filixic acid, and is unique in having an isobutyl bridge connecting the central acylphloroglucinol with two maximally methylated phloroglucinol residues, (syncarpic acid nuclei). Syncarpic acid itself was isolated by Diana *et al.*[6] who suspected it of being a degradation product of some parent compound. Since syncarpic acid was isolated from Syncarpia laurifolia belonging to the myrtaceae, our finding may suggest the parent compound to be one having a syncarpic acid nucleus connected by a c–c linkage to another moiety, possibly a phloroglucinol residue.

Apart from the acylphloroglucinols of the ferns, myrtucommulone is also related to the uliginosins[4,9] isolated from Hypericum uliginosum and the Kosins[10] isolated from Hagenia abyssinica.

The uliginosins[11] as well as the fern acylphloro-



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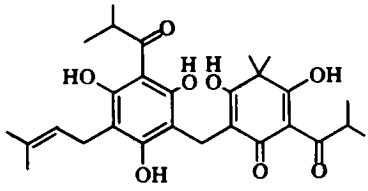


Ugliginosin—A

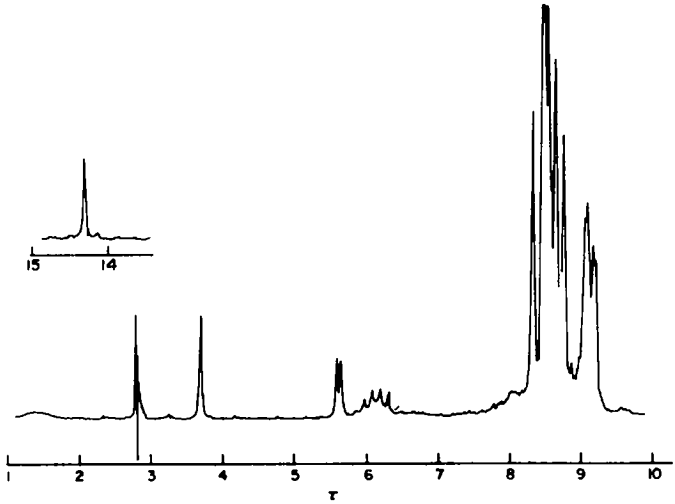
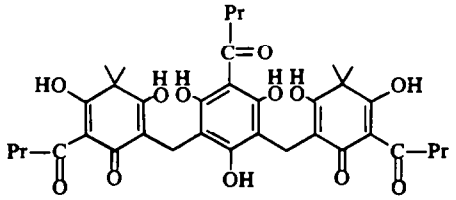
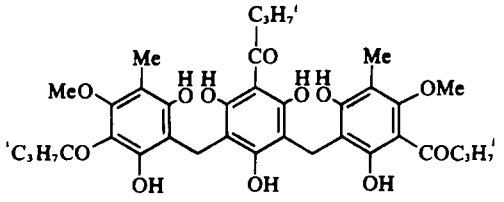


Fig 4. NMR spectrum of compound 2.



Filixic acid



Kosin (K_1)

glucinols are known to be antibacterial compounds. A further anology to myrtucommulone is the occurence of homologous mixtures in the fern acylphlotoglucinols[13,14] as well as in the uliginosins[4] and the kosins.[10]

Biosynthetically myrtucommulone could be formed similarly to the pathway suggested for the fern acylphloroglucinols,[15,16] namely through cyclization of linear polyketomethylen intermediates. Condensation of the rings thus obtained could possibly take place by a way analogous to that suggested for the synthesis of methylenebisphloroglucinols.[17] Isolation procedure and biological activity will be reported elsewhere.

EXPERIMENTAL

M.ps were taken on a Thomas Hoover capillary melting point apparatus, and are uncorrected. IR spectra were recorded on a Perkin-Elmer Infracord model 337 spectrophotometer. UV spectra were recorded on a Cary 14 spectrophotometer. NMR spectra were taken on a Varian HA-100 or JEOL JMN-C-60HL spectrometer using 5–10% solutions in $CDCl_3$, with TMS as an internal standard. Mass spectra were taken with an Hitachi Perkin-Elmer RMU-6 or AEI MS-902 high resolution mass spectrometer. TLC was performed on acidic (A) or basic (B) silica gel G plates[18] eluted with EtOAc and developed with 1% soln of Fast Blue salt-B (Fluka).

Myrtucommulone-A (1). Yellow crystals m.p. 185°–186° from MeOH, EtOAc or MeOH–$CHCl_3$ (Found: C, 68·37; H, 7·87; O, 23·89; $C_{38}H_{52}O_{10}$ requires: C, 68·24; H, 7·84; O, 23·92%); NMR ($CDCl_3$): δ 0·82 (12H), 1·22 (6H), 1·30–1·50 (24H), 3·06 m (2H), 3·82 m (2H), 4·61 septet (J = 7 Hz, 1H), 10·20–11·70 (3H), 13·15–13·35 (1H) and 16·30–16·70 (1H); ν_{max}^{KBr} 3500–2300 br. 1710, 1620, 1580, 1465, 1405, 1380, 1300, 1250, 1176, 1145, 1045, 1010 cm^{-1}. Mass spectrum: *m/e* 668 (43%), 607 (74%), 432 (9%), 389 (16%), 371 (60%), 301 (9%), 250 (12%), 236 (50%), 207 (60%), 196 (21%), 193 (35%), 166 (65%), 153 (100%) and 123 (80%); λ_{max}^{EtOH} 267 (18500) and 234 nm (22,000). TLC (B); R_f = 0·45 [brown].

Myrtucommulone-B (2). Amorphous powder; (Found: C, 69·35; H, 7·59; O, 23·05; $C_{24}H_{30}O_6$ requires: C-69·54; H, 7·30; O, 23·16%); NMR ($CDCl_3$): δ 0·80 d and 0·82 d (J = 6·5 Hz, 6H), 1·25 d (J = 6·5 Hz, 6H), 1·38s (3H), 1·43s (6H), 1·62s (3H), 1·94 m (1H) 3·92 septet (J = 6·5 Hz, 1H), 4·31 d (J = 3 Hz, 1H) and 6·26s (1H); Mass spectrum: *m/e* 371 (100%), 301 (5·5%) 70 (4%); ν_{max}^{KBr} 3330 (OH), 2930, 1715, (CO), 1650, 1600, 1150, 1035, 990, 840, 750 cm^{-1}. TLC (B): $R_f \sim 0·8$ (red).

Alkaline cleavage of compound 1. Compound 1 (400 mg) in 10% NaOH aq (50 ml) was refluxed for 3–5 h. After cooling the soln was acidified with HCl and extracted several times with ether. The dried ether (Na_2SO_4) was evaporated and the residue crystallized from EtOAc-cyclohexane to give syncarpic acid, m.p. 190°, $CHCl_3$, lit 190°.[10] (Found: C, 65·89; H, 7·82; O, 26·89; $C_{10}H_{14}O_3$ requires: C, 65·92; H, 7·74; O, 26·34%); NMR ($CDCl_3$): δ 1·35s and 1·40s (12H)—pH dependent (see Ref 5) 3·6s (~ 1H). Mass spectrum: *m/e* 182 (72%, M^+), 154 (7·5%, M-CO), 112 (11%, M-70), 70 (100% O=C=C $(CH_3)_2$); ν_{max}^{KBr} 3100–2500 br., 1700, 1620, 1530, 1480, 1310, 1220, 1250, 1130, 1040, 940, 860, 810 cm^{-1}; TLC (B); R_f 0·1 (red-orange). The syncarpic acid was further identified as the OMe derivative[6] prepared in the usual way with Me_2SO_4; NMR ($CDCl_3$): 1·32s (6H), 1·38s (6H), 3·78s (3H) and 5·5s (1H). Mass spectrum: *m/e* 196 (33%, M^+), 126 (100%, M-70), 125 (10%), 111 (10%) and 70 (12%, O=C=C $(CH_3)_2$).

The above neutralized aqueous phase was evaporated to dryness under reduced pressure and the residue extracted with boiling acetone. The acetone was then evaporated and the residue crystallized from EtOAc–$CHCl_3$; m.p. 205°, mixed m.p. with phloroglucinol (BDH) unchanged; TLC (B), $R_f \sim 0·45$ (violet-blue).

Acidic treatment of compound 1 *to yield* 3. Compound 1 (100 mg) was refluxed in benzene (50 ml) in the presence of *p*-TsOH (10 mg) for 5 h, or in EtOH-HCl (10; 1) (10 ml) for the same time. Following the usual workup the residue was crystallized from cyclohexane; m.p. 290°–295° dec (Found: C, 72·50; H, 8·00; O, 19·85; $C_{38}H_{48}O_8$ requires: C, 72·13; H, 7·65; O, 20·23%); NMR ($CDCl_3$): δ 0·80 d (J = 6·5 Hz, 12H), 1·22 d and 1·24 d (J = 6·5 Hz, 6H), 1·45 br.s(18H), 1·54 s (6H), 2·00 m (2H), 3·12 septet (J = 6·5 Hz, 1H), 4·63 d (J = 3·5 Hz, 2H) and 8·40 brs (1H). Mass spectrum: *m/e* 632 (< 0·1%, M^+), 589 (100%, M-iPr), 519 (19%, M—O=C=C$(CH_3)_2$), 461 (15%); ν_{max}^{KBr} 3350(OH), 2950, 2910, 1710(CO), 1650, 1600, 1170, 980 cm^{-1}.

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