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Monoterpenes of *Teucrium marum*

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Abstract

Four cyclopentanoid monoterpenes were isolated from *Teucrium marum*; they are the two [(A) and (B)] C2-epimers of α-(2-formyl-3-methylcyclopentyl)acrylaldehyde and two new [(C) and (D)] isomeric unsaturated lactones. Structures were established by spectral (u.v., i.r., n.m.r., m.s.) evidence and chemical transformations.

Teucrium marum (Labiatae) is a wild plant that grows in the Mediterranean area and is characterized by a powerful lacrimatory essential oil.

Four monoterpenoid components (A), (B), (C) and (D) (9 : 1 : 2 : 38) were obtained in 0·05% yields by hexane extraction; subsequently, chloroform extracted (0·65%) only the products (A) and (B) in a mixture (9 : 1) which can be purified by steam distillation but decomposes slowly at room temperature.

Structures of (A) and (B)

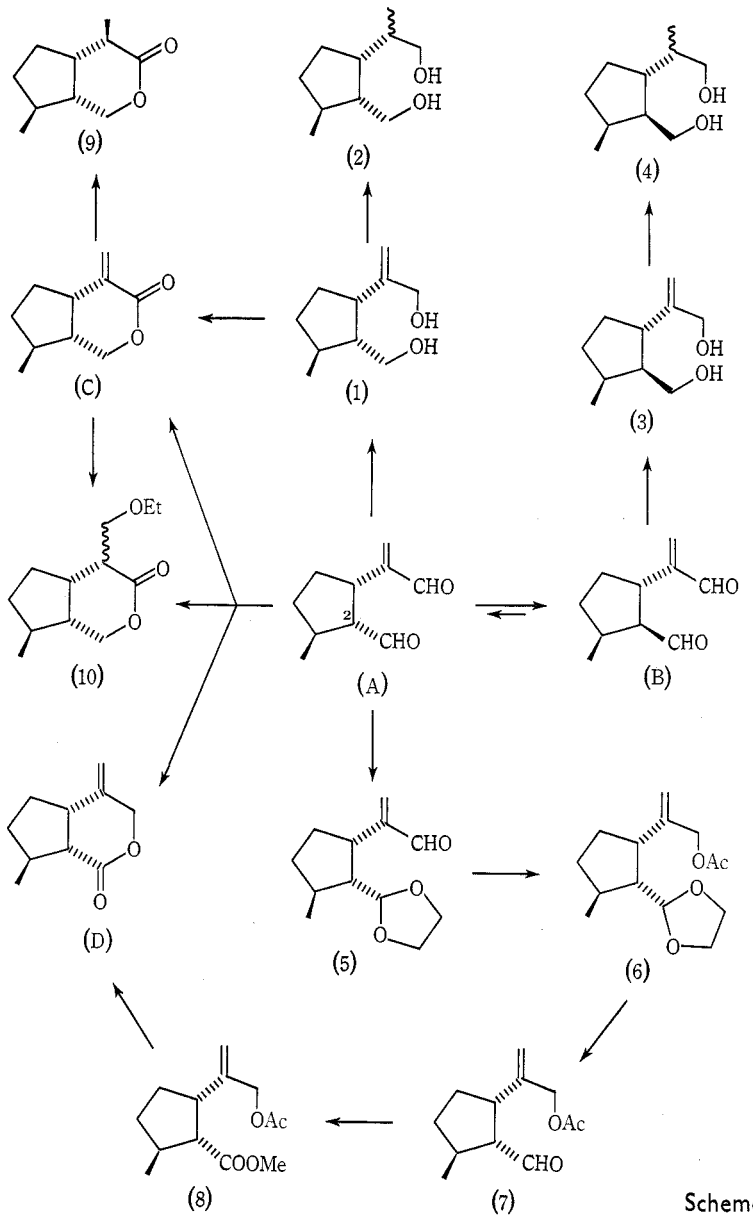
Repeated micropreparative g.l.c. of the chloroform extract isolated (94–96% pure) the components (A), $[\alpha]_D^{20}$ −72·0°, and (B), $[\alpha]_D^{20}$ +3·5°, which showed (g.c.–m.s.) an identical molecular ion (M^+ 166) and a perfectly superimposable fragmentation pattern.

Treatment of both substances with sodium methoxide afforded a equilibrium mixture (1 : 3) of (A) and (B). While their i.r. and u.v. absorptions are identical, their n.m.r. spectra differ, mainly with regard to the chemical shifts of the aldehydic protons: δ 9·38 and 9·51 for (A) and at δ 9·52 and 9·76 for (B). The spectral data, together with elementary analysis ($C_{10}H_{14}O_2$), suggested that they were C2-epimers of α-(2-formyl-3-methylcyclopentyl)acrylaldehyde. These compounds had been previously isolated by Cavill and Hinterberger[1] from some species of *Dolichoderus* ants (dolichodial, a non-separable mixture) and by Meinwald *et al.*[2] from a phasmid insect (anisomorphal*).

* On account of the physical and spectral data reported for anisomorphal, we believe it to be the pure isomer (B).

[1] Cavill, G. W. K., and Hinterberger, H., *Aust. J. Chem.*, 1960, **13**, 514; 1961, **14**, 143.
[2] Meinwald, J., Chadha, M. S., Hurst, J. J., and Eisner, T., *Tetrahedron Lett.*, 1962, **1**, 29.

The stereochemistry of (A) and (B) was assigned on the basis of the transformations reported in Scheme 1. On $NaBH_4$ reduction* and subsequent hydrogenation†



Scheme 1

* Catalytic reduction followed by oxidation to nepetalinic acids served no useful purpose, for the intermediate saturated aldehyde equilibrates.[3]

† Meinwald *et al.*[2] report that hydrogenolysis products are formed in the reduction of dolichodial, but no experimental data are given. In our reduction, hydrogenolysis processes are negligible.

[3] Cavill, G. W. K., and Ford, D. L., *Aust. J. Chem.*, 1960, **13**, 296.

(PtO_2–benzene), the aldehyde (A) gives saturated diols (2) with g.l.c. retention times[4] identical to those of authentic '*cis-trans*'[5] iridodiols.

The same reactions, carried out on aldehyde (B), afforded the diols (4) with g.l.c. retention times[4] corresponding to those of authentic '*trans-cis*'[5] isomers.

Accordingly, it is now possible to assign the configurations to the two derivatives of 2,4-dinitrophenylhydrazine with dolichodial isolated by Cavill and Hinterberger,[1] the red one, m.p. 239–242°, corresponding to (A), and the yellow one, m.p. 176–177, corresponding to (B).

Structures of (C) and (D)

Silica gel chromatography of the hexane extract gave a mixture of (C) and (D), which showed (g.c.–m.s.) an identical molecular ion (M^+ 166) and very similar fragmentation patterns. The mixture slowly dissolves in dilute alkali and may be recovered after acidification.

On the basis of elementary analysis ($C_{10}H_{14}O_2$), i.r. and n.m.r. spectra, structure (D) was assigned to the most abundant component, $[\alpha]_D^{20}$ +31·7°, separated by preparative g.l.c. in 95% purity; this structure was confirmed by synthesis from aldehyde (A) (Scheme 1). Selective transacetalation of (A) with 2-ethyl-2-methyl-1,3-dioxolan[6] leads to (5), from which the ester (8) was obtained as shown. Alkaline hydrolysis of (8) and treatment with acid afforded a lactone with physical and spectral data identical to those of the natural compound (D), for which we propose the name of dolicholactone.

The structure of (C), which we named allodolicholactone, was determined from a sample consisting of 80% of (C) and 20% of (D); no higher purity could be attained owing to the small amounts available.

Comparison of the n.m.r. spectrum of this mixture with that of the lactone (D) suggested that the structure (C) might be assigned to this compound; this was then confirmed by synthesis. When diol (1) was treated with silver carbonate/Celite[7] a homogeneous (t.l.c. and g.l.c.) compound, $[\alpha]_D^{20}$ +145·3°, was obtained in good yield; its n.m.r. and i.r. spectra coincided perfectly with those deduced for (C), as did the g.l.c. retention time and u.v. absorption.

When (C) was submitted to catalytic hydrogenation with 5% $Pd/CaCO_3$ in hexane, iridomyrmecin (9), $[\alpha]_D^{20}$ +203·5°,[8] was obtained as a pure isomer in nearly quantitative yields. On account of this conversion, the absolute configurations shown in the reported formulae may be assigned to the monoterpenes (A), (B), (C) and (D) for *T. marum*.

A Cannizzaro-type reaction, already proposed for iridolactones,[9] might account for the biosynthesis of (C) and (D).*

* The hypothesis that the lactonic components of *T. marum* might be an artefact formed on handling or on storage was rejected because (C) and (D) were detected in freshly gathered plants and were not found among the products obtained from the alteration of (A) and (B).

[4] Regnier, F. E., Waller, G. R., and Eisenbraun, E. J., *Phytochemistry*, 1967, **6**, 1281.
[5] Cavill, G. W. K., and Whitfield, F. B., *Aust. J. Chem.*, 1964, **17**, 1260.
[6] Dauben, H. J., Loken, B., and Ringold, H. J., *J. Am. Chem. Soc.*, 1954, **76**, 1359.
[7] Fétizon, M., and Golfier, M., *C. R. Acad. Sci.*, 1968, **267**, 900.
[8] Fusco, R., Trave, R., and Vercellone, A., *Chim. Ind. (Milan)*, **37**, 251.
[9] Clark, K. J., Fray, G. I., Jaeger, R. H., and Robinson, R., *Tetrahedron*, 1959, **6**, 217.

This hypothesis was chemically tested. Treatment of (A) with alkali in ethanol[3] or methanol afforded a mixture of (10) (80%), (C) (15%) and (D) (5%); in water[10] (C) and (D) (5 : 2) were obtained in low yields (16%). The product of vinylogous nucleophilic addition (10) was proved to derive from (C) in the reaction conditions. The ratio between the unsaturated lactones (C) and (D), which were present in very small amounts in the mixture, turned out to be the opposite of that found in the plant, thus confirming that they are not artefacts.

A relatively greater quantity of (C) in the Cannizzaro reactions may be explained if it is borne in mind that the saturated aldehyde is enolized by alkali, thus preventing the formation of its hydrate and the subsequent detachment of hydride anion.

To sum up, it is remarkable that dolichodial, which had previously been found in insects 'widely separated phylogenetically from one another'[2] (ants and phasmids), should also occur in the vegetable kingdom.

Experimental

Melting points were determined on a Tottoli block and are uncorrected. Infrared spectra were determined with a Perkin–Elmer 257 instrument. N.m.r. spectra were measured on a Jeol C-60 instrument, with tetramethylsilane as internal standard; chemical shifts have been recorded as δ values. Mass spectra were determined with a Varian MAT 112 spectrometer. Ultraviolet spectra were recorded on a Beckman DB-GT grating spectrophotometer. Optical rotations were measured on a Perkin–Elmer 141 instrument.

A herbarium voucher of *T. marum* is deposited in the Botanical Institute of the University of Modena (No. 7546/28).

Extraction and Isolation of Monoterpenes

Fresh plants (11 kg) of *T. marum*, collected in Sardinia in June, were extracted in a column successively with hexane (60 l.) and chloroform (60 l.).

(A) After evaporation of the hexane, the residual oil (28 g) was repeatedly chromatographed on silica gel, hexane–ether being used as eluent. A mixture (3·5 g) of (C) and (D) (1 : 19), b.p. 110–113°/0·5 mm, $[\alpha]_D^{20}$ +33·3° (*c*, 4 in benzene), was thus obtained. Subsequent preparative g.l.c. (NPGS 5% on Chromosorb W, 170°) separated *component* (*D*) (95% pure), $[\alpha]_D^{20}$ +31·7° (*c*, 4·5 in benzene) [i.r. (CCl_4): 1730 cm^{-1}; n.m.r. (CCl_4): δ 1·17 (3H, d, *J* 6·5 Hz, CH_3–C); 3·0 (1H, m, CH–C=); 4·5 (2H, s, CH_2–O); 4·95 and 5·05 (1H each, m, CH=C) (Found: C, 72·4; H, 8·4. $C_{10}H_{14}O_2$ requires C, 72·3; H, 14·1%)] and a mixture (4 : 1) of (C) and (D), $[\alpha]_D^{20}$ +122·0° (*c*, 4·1 in benzene) [i.r. (CCl_4): 1733 cm^{-1}; u.v. (MeOH): λ_{max} 238 nm; n.m.r. (CCl_4): in addition to the signal of (D), δ 1·12 (3H, d, *J* 6 Hz, CH_3–C); 4·1 (2H, m, CH_2OCO); 5·38 and 5·90 (1H each, t, *J* 1·5 Hz, CH=C)].

(B) The chloroform residue (136 g) was steam distilled and the distillate extracted with chloroform, after the addition of NaCl. Evaporation of the solvent and distillation (80–82°/0·5 mm) gave a yellow mixture (71 g) of components (A) and (B) (9 : 1), which was used in the isolation, by preparative g.l.c. (NPGS 5% on Chromosorb W, 120°), of the (A) component, $[\alpha]_D^{20}$ −72·0° (*c*, 4·2 in benzene); i.r. (CCl_4): 2710, 1724, 1695, 1630 and 950 cm^{-1}; u.v. (H_2O): λ_{max} 223 nm (ε 6965); n.m.r. ($CDCl_3$): δ 1·08 (3H, d, *J* 6·5 Hz, CH_3–C); 3·3 (1H, m, CH–C=); 6·12 (1H, s, CH=C); 6·28 (1H, d, *J* 1·4 Hz, CH=C); 9·38 (1H, d, *J* 2·5, –C–CHO) and 9·51 (1H, s, =C–CHO) (Found: C, 72·5; H, 13·9. Calc. for $C_{10}H_{14}O_2$: C, 72·3; H, 14·1%). 2,4-Dinitrophenylhydrazone (red), m.p. 239–242° (dimethylformamide) (lit.[1] 242°).

The above mixture (5 g) was treated with 0·1 M methanolic sodium methoxide (40 ml) at room temperature for 2 h (g.l.c. check) and then quenched with dil. H_2SO_4. Extraction ($CHCl_3$) and distillation afforded a mixture (1 : 3) of (A) and (B), from which the component (B) was isolated by preparative g.l.c., $[\alpha]_D^{20}$ +3·5° (*c*, 4·3 in benzene); i.r. (CCl_4): 2710, 1722, 1696, 1630 and 950

[10] Cavill, G. W. K., Ford, D. L., and Locksley, H. D., *Aust. J. Chem.*, 1956, **9**, 288.

cm^{-1}; u.v. (H_2O): λ_{max} 223 nm (ε 6930); n.m.r. ($CDCl_3$): δ 1·07 (3H, d, J 6·5 Hz, CH_3–C); 3·4 (1H, m, CH–C=); 6·0 and 6·3 (1H each, s, CH=C); 9·52 (1H, s, =C–CHO) and 9·76 (1H, d, J 2·6, –C–CHO) (Found: C, 72·0; H, 14·3. Calc. for $C_{10}H_{14}O_2$: C, 72·3; H, 14·1%). 2,4-Dinitrophenylhydrazone (yellow), m.p. 176–177° (benzene) (lit.[1] 177°)

'cis-trans' *Iridodiols* (*2*)

A solution of (A) (1·4 g) in methanol (25 ml) was treated with $NaBH_4$ (1·7 g) at room temperature. Usual workup afforded the *unsaturated diol* (1) (1·19 g) as a pure (g.l.c.) oil, $[\alpha]_D^{20}$ −5·79° (*c*, 14 in CCl_4); i.r. (film): 3300 cm^{-1}; n.m.r. (CCl_4): δ 1·08 (3H, d, J 6 Hz, CH_3–C); 2·7 (1H, m, CH–C=); 3·36 (2H, m, –C–CH_2–O); 4·0 (2H, s, =C–CH_2–O); 4·82 and 5·1 (1H each, br s, CH=C) (Found: C, 70·6; H, 10·5. $C_{10}H_{18}O_2$ requires C, 70·6; H, 10·7%). Compound (1) (100 mg) was dissolved in benzene and hydrogenated (1 mol H_2, room temperature and pressure) in the presence of PtO_2. The solution was then filtered and evaporated to yield (2) as a colourless oil which was purified by distillation; i.r. ($CHCl_3$): 3300 cm^{-1}; n.m.r. ($CDCl_3$): δ 0·98 (6H, d, J 6 Hz, CH_3–C) and 3·37 (4H, m, CH_2–O). The g.l.c.[4] retention times correspond to those of authentic '*cis-trans*' iridodiols.

'trans-cis' *Iridodiols* (*4*)

The above procedures carried out on (B) (0·11 g) afforded the isomeric *unsaturated diol* (*3*), [m.p. 35–36° (pentane), $[\alpha]_D^{20}$ 0° (*c*, 14 in CCl_4); i.r. (film): 3300 cm^{-1} (Found: C, 70·4; H, 10·8. $C_{10}H_{18}O_2$ requires C, 70·6; H, 10·7%)] and then (4) (oil), purified by distillation (51 mg); i.r. ($CHCl_3$): 3300 cm^{-1}; n.m.r. ($CDCl_3$): δ 0·99 (6H, d, J 6 Hz, CH_3–C) and 3·37 (4H, m, CH_2–O). Compound (4) had the same g.l.c.[4] retention times as authentic '*trans-cis*' iridodiols.

α-[c-2-(1,3-Dioxolan-2-yl)-t-3-methylcylcopent-r-1-yl]acrylaldehyde (*5*)

Compound (A) (1 g) was dissolved in benzene (50 ml) and treated with 2-ethyl-2-methyl-1,3-dioxolan[6] (2·8 g) and *p*-toluenesulphonic acid (50 mg). The mixture was treated under reflux for 48 h and g.l.c. showed almost complete conversion of starting material. The reaction mixture was cooled, treated with solid Na_2CO_3 and filtered over silica gel. The solvent and the excess of reagent were evaporated under vacuum (10 mm), affording the acetal (5)* as a pale yellow oil (0·90 g) which proved to be unstable on distillation (0·02 mm) and on chromatography; i.r. (CCl_4): 2700, 1690, 1630 and 940 cm^{-1}; u.v. (H_2O): λ_{max} 223 nm (ε 5890); n.m.r. (CCl_4): δ 1·08 (3H, d, J 6 Hz, CH_3–C); 3·03 (1H, m, CH–C=); 3·7 (4H, m, CH_2–O); 4·37 (1H, d, J 4·2 Hz, O–CH–O); 5·92 and 6·1 (1H each, br s, CH=C) and 9·62 (1H, s, CH=O).

α-[c-2-(1,3-Dioxolan-2-yl)-t-3-methylcyclopent-r-1-yl]allyl Acetate (*6*)

Crude (5) (2·5 g) was dissolved in ethanol (50 ml) and treated with sodium borohydride (1·2 g). Usual workup afforded an unstable oily alcohol [i.r. (film): 3300 cm^{-1}] which was directly acetylated with acetic anhydride (3 ml) and pyridine (2·5 ml). The mixture was fractionated by distillation to give the *acetate* (6) (2·2 g), b.p. 150–160°/0·3 mm; i.r. (CCl_4): 1735, 1650 and 1230 cm^{-1}; n.m.r. (CCl_4): δ 1·06 (3H, d, J 6 Hz, CH_3–C); 2·01 (3H, s, CH_3COO); 2·6 (1H, m, CH–C=); 3·82 (4H, m, O–$(CH_2)_2$–O); 4·5 (2H, s, CH_2–O); 4·75 (1H, d, J 3·7 Hz, O–CH–O); 4·92 and 5·04 (1H each, br s, CH=C) (Found: C, 66·0; H, 9·0. $C_{14}H_{22}O_4$ requires C, 66·1; H, 8·7%).

α-(c-2-Formyl-t-3-methylcyclopent-r-1-yl)allyl Acetate (*7*)

The acetal (6) (1·48 g) was dissolved in acetone (30 ml) and treated with water (20 ml) and *p*-toluenesulphonic acid (70 mg) at 60° for 1 h. The reaction mixture was cooled, diluted with water (40 ml) and extracted with methylene chloride (3 × 20 ml). The combined extracts were evaporated to give, after distillation (100–104°/0·1 mm), the *acetate* (7) (oil) (0·8 g); i.r. (CCl_4): 2720, 1735, 1720, 1650 and 1240 cm^{-1}; n.m.r. (CCl_4): δ 1·1 (3H, d, J 6 Hz, CH_3C); 2·07 (3H, s, CH_3COO);

* A limited (10–12%) inversion at C2 (g.l.c., t.l.c.) accompanied the transformation of (A) to (8); the final ring closure to (D), however, removed the minor isomer.

2·7 (1H, m, CH–C=); 4·57 (2H, br s, CH_2–O); 5·03 and 5·1 (1H each, br s, CH=C) and 9·67 (1H, d, *J* 3 Hz, C–CHO) (Found: C, 68·3; H, 8·7. $C_{12}H_{18}O_3$ requires C, 68·5; H, 8·6%).

α-(c-2-Methoxycarbonyl-t-3-methylcyclopent-r-1-yl)allyl Acetate (8)

The acetate (7) (0·7 g) was dissolved in acetone (20 ml) and Jones reagent added dropwise at 0°. Usual workup afforded an acidic unstable compound which was treated with a slight excess of ethereal diazomethane to give, after distillation (98–102°/0·15 mm), the *ester* (8) (0·5 g); i.r. (CCl_4): 1735, 1715, 1650 and 1240 cm^{-1}; n.m.r. (CCl_4): δ 1·08 (3H, d, *J* 6 Hz, CH_3–C); 2·02 (3H, s, CH_3COO); 2·6 (1H, m, CH–C=); 3·51 (3H, s, CH_3–O); 4·47 (2H, s, CH_2–O); 4·95 and 5·05 (1H each, br s, CH=C) (Found: C, 64·7; H, 8·5. $C_{13}H_{20}O_4$ requires C, 65·0; H, 8·4%).

Lactonization of (8)

The ester (8) (0·5 g) was treated with 10% aqueous NaOH (10 ml) at 90° for 30 min. After acidification with 20% HCl, the mixture was cooled and then extracted with ether (3 × 20 ml). The combined extracts were washed with 10% $NaHCO_3$ solution and then evaporated. The oily residue was distilled (90–94°/0·1 mm) to give pure (g.l.c., t.l.c.) (D) (0·23 g), $[\alpha]_D^{20}$ +31·4° (*c*, 4·1 in benzene). The spectral data were found to be identical to those of the natural product.

Oxidation of (1) to (C)

The silver carbonate/Celite reagent[7] (30 g) was suspended in a benzene solution (250 ml) of the alcohol (1) (1·09 g); 100 ml of benzene is distilled azeotropically and then the solution is refluxed. After 2 h, the solid is filtered off and the lactone isolated by evaporation of the benzene and chromatography over silica gel. Elution with benzene–acetone gradient afforded pure *lactone* (*C*) (oil) (417 mg), $[\alpha]_D^{20}$ +145·3° (*c*, 4·5 in benzene); i.r. (CCl_4): 1733 and 1630 cm^{-1}; u.v. (MeOH): λ_{max} 238 nm (ε 2·4 × 10^4); n.m.r. (CCl_4): δ 1·12 (3H, d, *J* 6 Hz, CH_3–C); 3·2 (1H, m, CH–C=); 4·1 (2H, m, CH_2OCO); 5·38 and 5·90 (1H each, t, *J* 1·5 Hz, CH=C) (Found: C, 72·2; H, 8·6. $C_{10}H_{14}O_2$ requires C, 72·3; H, 8·5%). The g.l.c. retention time was identical to that of the natural product.

Hydrogenation of (C)

The lactone (C) (104 mg), obtained by synthesis from (1), was dissolved in hexane (10 ml) and hydrogenated in the presence of 5% $Pd/CaCO_3$ (100 mg) at room temperature and pressure. After 5 min the reaction was complete (g.l.c.) and usual workup afforded an oil (94 mg), homogeneous on t.l.c. and g.l.c., which solidified on standing. Crystallization from n-pentane gave pure iridomyrmecin (9), m.p. 62–63°, undepressed on admixture with an authentic sample, $[\alpha]_D^{20}$ + 203·5°[8] (*c*, 4 in EtOH). The i.r. and n.m.r. spectra are superimposable with those of the natural product.

Reaction of (A) with Alkali

(A) *In ethanol.*[3]—The natural mixture (9 : 1) of (A) and (B) (537 mg) was dissolved in ethanol (45 ml) and treated with 10% aqueous potassium hydroxide (5 ml) under reflux. After 2 h, the solution was cooled and the alcohol removed in vacuum. Dilution with water (30 ml) and extraction with $CHCl_3$ gave a neutral fraction (42 mg). The aqueous solution was acidified with excess of HCl (pH 4) and then extracted with $CHCl_3$ (3 × 20 ml). The combined extracts, dried (Na_2SO_4), gave on evaporation a mixture (408 mg) of (10), (C) and (D) (80 : 15 : 5). The components were separated by repeated chromatography over silica gel, benzene–ether mixtures being used as eluents. The spectral data of (C) and (D) were identical to those of natural products. The ethoxymethyl lactones (10) were further purified by crystallization (n-pentane), m.p. 68°, $[\alpha]_D^{20}$ −18·9° (*c*, 4·2 in benzene); i.r. (CCl_4): 1740 cm^{-1}; n.m.r. (CCl_4): 0·85–1·4 (6H, m, CH_3–C); 3·2–3·7 (4H, m, CH_2–O) and 3·7–4·4 (2H, m, CH_2OCO); mass spectrum: *m/e* 212 (M^+), 183 ($M^+ - C_2H_5$) (Found: C, 68·0; H, 9·6. Calc. for $C_{12}H_{20}O_3$: C, 67·9; H, 9·5%).

(B) *In water.*[10]—The reaction carried out in water gave mostly pitch-like products and a small quantity (15–17% yield) of (C) and (D) (5 : 2).

Compound (10) from (C)

The lactone (C) (200 mg) was dissolved in ethanol (20 ml) and treated with 10% aqueous potassium hydroxide (2·2 ml) under reflux (2 h). Workup as described above afforded (10) (150 mg), m.p. 68–69°, with physical and spectral data identical to those previously reported.

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