Isolation and Structure Determination of a Novel Phorbol Derivative in an Intramolecular Diester Macrolide

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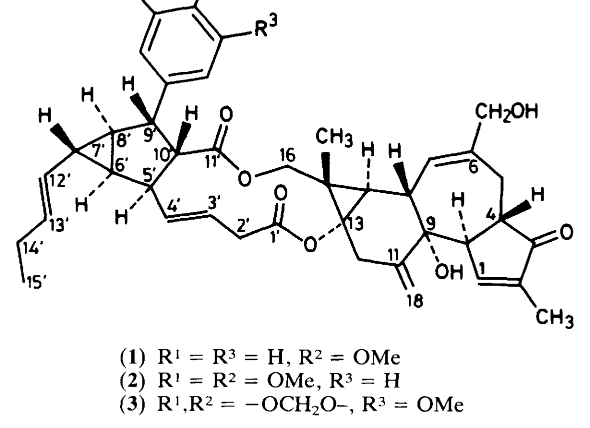
Three proteinkinase C-agonizing macrolides, formed by esterification of the novel phorbol derivative 11,18-dehydro-4,12-dideoxy-16-hydroxyphorbol with dicarboxylic acids, that differ only in the state of oxidation of a phenyl residue, were isolated from the dried leaves of *Pycnocoma cornuta* Muell. (Euphorbiaceae), and their structures were determined by n.m.r. studies.

Pycnocoma cornuta Muell. (Euphorbiaceae), a low erect shrub used by local herbalists in Nigeria as a purgative and an antiemetic, is currently being investigated in order to identify its pharmacologically active components. An earlier study of this plant showed that an extract of the root has antitumour activity in the P388 lymphocytic leukemia test screen and that scopoletin, the only substance identified, was partly responsible for this effect.[1] In the course of our present work, four highly active proteinkinase C-agonizing[2] compounds have been isolated, (*ca.* 20 mg of each compound from 1 kg of dried leaves), and the structure determined for three of them. Extensive n.m.r. studies and high resolution mass spectrometry data showed the structures to be (**1**), (**2**), and (**3**).† This is the first 11,18-dehydrophorbol derivative reported, and also the first 4,12-dideoxy-16-hydroxyphorbol derivative



(1) $R^1 = R^3 = H$, $R^2 = OMe$
(2) $R^1 = R^2 = OMe$, $R^3 = H$
(3) $R^1,R^2 = -OCH_2O-$, $R^3 = OMe$

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† Selected spectral data. Pycnocomolide 4″-methoxy- (**1**), $[\alpha]_D$ +124° (*c* 1.0 in MeOH); fast atom bombardment mass spectrometry (f.a.b.-m.s.) (*m*-nitrobenzyl alcohol) m/z 681.3496 (MH^+) ($C_{42}H_{49}O_8$ requires 681.3427); u.v. (EtOH) $\lambda_{max.}$ (ε), 226 (16 000), 276 (1300) and 284 nm (1100); i.r. (KBr) $\nu_{max.}$ 3410, 2960, 2920 br, 1740—1690 br, 1630, 1510, 1460, 1440, 985, and 965 cm^{-1}. Pycnocomolide 3″,4″-dimethoxy- (**2**), $[\alpha]_D$ +150° (*c* 1.0 in MeOH); f.a.b.-m.s. (*m*-nitrobenzyl alcohol) m/z 710.3470 (M^+) ($C_{43}H_{50}O_9$ requires 710.3455); u.v. (EtOH) $\lambda_{max.}$ (ε), 228 (infl.) and 277 nm (3200); i.r. (KBr) $\nu_{max.}$ 3410, 2960, 2930, 1740—1690 br, 1630, 1510, 1440, 1420, 985, and 965 cm^{-1}. Pycnocomolide 5″-methoxy-3″,4″-methylenedioxy- (**3**), $[\alpha]_D$ +129° (*c* 1.0 in MeOH); f.a.b.-m.s. (*m*-nitrobenzyl alcohol) m/z 724.3312 (M^+) ($C_{43}H_{48}O_{10}$ requires 724.3248); u.v. (EtOH) $\lambda_{max.}$ (ε), 238 (infl.), 273 (infl.), and 283 nm (infl.); i.r. (KBr) $\nu_{max.}$ 3410, 2960, 2920, 1740—1690 br, 1630, 1510, 1450, 1430, 985, and 965 cm^{-1}.

known. The dicarboxylic acid part of the structures has a novel carbon skeleton.

^1H–^1H two-dimensional n.m.r. spectroscopy, a P.E.COSY[3] experiment, gave all, except one (discussed below), of the apparent $^3J_{HH}$ correlations, as well as the magnitudes of the coupling constants. In addition, all geminal and several long-range couplings were well resolved. No coupling between the vicinal protons H-8′ and H-9′ was observed. However, molecular mechanics calculations‡ of the structures gave low-energy conformations of the bicyclo[3.1.0]hexane ring system with a dihedral angle of about 80° for the two carbon–proton bonds in question. The Karplus equation, modified according to Haasnoot *et al.*,[4] gave an expected $^3J_{HH}$ coupling constant of <1 Hz for that angle.

^{13}C–^1H two-dimensional n.m.r. spectroscopy, optimized for direct as well as for long-range couplings,[5] was used together with one-dimensional ^{13}C–^1H correlation techniques, in order to establish the connectivity in the carbon skeleton, *i.e.* identification of quaternary and oxygen-carrying carbon atoms. The existence of two three-membered rings was confirmed by their large $^1J_{CH}$ coupling constants, ranging from 158 to 171 Hz.

Compounds (**1**), (**2**), and (**3**), which are all amorphous, are derivatives of a common hypothetic diester marcrolide, which we call pycnocomolide. The corresponding hypothetical diacid, carrying no substituents in the phenyl residue, is named pycnocomic acid. None of (**1**), (**2**), or (**3**) has as yet been degraded chemically or subjected to *X*-ray crystallography. Consequently, the absolute configuration of the pycnocomic acid part of the pycnocomolides is still unknown. Lack of a reference system containing the aromatic ring has precluded the use of c.d. spectroscopy for confirmation of the absolute configuration of the phorbol part.

Two-dimensional homonuclear Overhauser enhancement (n.O.e.) spectroscopy, NOESY, with a 16-step phase cycle,[6] gave a chart of correlations well in accord with the internuclear distances found in a molecular mechanics‡ calculation. The relative configuration of the bicyclo[3.1.0]hexane moiety, in part suggested by the measured $^nJ_{HH}$ coupling constants, was confirmed by the NOESY correlations. The relative configuration of the phorbol part, which is the same as has been found in other naturally occurring 4-deoxyphorbol derivatives,[7] was similarly established. No dipole–dipole interaction was found between the phorbol and the pycnocomic acid moiety, relating their relative configurations. All n.m.r. studies have been done on a Varian XL-300 n.m.r. spectrometer, operating at 299.9 MHz for ^1H and 75.4 MHz for ^{13}C.§

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‡ Energy-minimized geometries of the pycnocomolides were calculated using the MM2(85) molecular mechanics program[8] with an extended set of force field parameters. The calculations were performed for two of the diastereoisomers of each pycnocomolide in such a way that the phorbol was kept in one configuration and the pycnocomic acid moiety altered in its two enantiomeric forms. The conformers of the bicyclo[3.1.0]hexane and the phorbol ring system thus generated were similar regarding atomic distances and the dihedral angles that would affect the n.m.r. data.

§ After submitting this paper, we became aware that a related macrocyclic diester between 12-deoxy-16-hydroxyphorbol and a nonaromatic diacid containing a bicyclo[3.1.0]hexane unit has been isolated from *Jatropha curcas* L.[9]