EXPERIENTIA

Vol. 31 – Fasc. 2 Pag. 137 – 264 15. 2. 1975

SPECIALIA

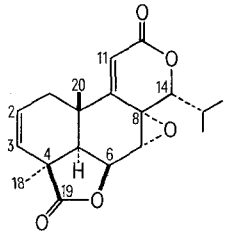
Podolide, a New Antileukemic Norditerpene Dilactone from *Podocarpus gracilior*[1]

In recent years a number of norditerpenoid lactones have been isolated from both plant and fungal sources[2]. In the course of a continuing search for tumor-inhibitory compounds from higher plants we have isolated a new member of this series from *Podocarpus gracilior* Pilg. (Taxaceae). Podolide (**1**) is the first compound of this class reported to show tumor-inhibitory activity[3].

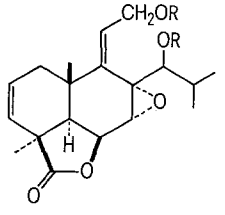
The tumor-inhibitory activity of an ethanol extract of the twigs and leaves of *P. gracilior* was concentrated in the chloroform layer of a chloroform-water partition and then in the aqueous methanol layer of an aqueous methanol-Skellysolve B partition. Successive column chromatography on silica gel and SilicAR CC-7, guided by assay against P-388 cell culture, yielded podolide (**1**) (0.0025%), $C_{19}H_{22}O_5$; mp 296–298°; $[\alpha]_D^{25}$–12° (*c* 0.66, pyridine)[4]. Absorption in the UV at 218 nm (ε 13,000) and IR at 5.87 μ (KBr) suggested the presence of an α,β-unsaturated δ-lactone, while a second IR band at 5.63 μ was indicative of a γ-lactone moiety.

The similarity of these data to those described for inumakilactone A[5] and structurally similar compounds[2] suggested that podolide possessed a norditerpenoid skeleton. This was confirmed by comparison of the PMR-spectrum of (**1**) to published data. Thus signals at τ 4.01 (1H, s), 5.05 (1H, dd, J 4.4, 1.6 Hz), 6.06 (1H, d, J 1.6 Hz), and 8.16 (1H, d, J 4.4 Hz) could be assigned to the 11-H, 6-H, 7-H and 5-H, respectively.

Reduction of podolide with sodium borohydride afforded the oily diol (**2**) as the major product[6]. The PMR-spectrum of (**2**), in contrast to the complexity of the methyl region in the spectrum of (**1**), showed clearly-discernible signals corresponding to two angular methyl groups (τ 8.80, 8.97; 6H, 2s) and an isopropyl group (τ 8.94, 9.08; 6H, 2d, J 7.0 Hz). Oxidation of (**2**) with manganese dioxide gave podolide in excellent yield.

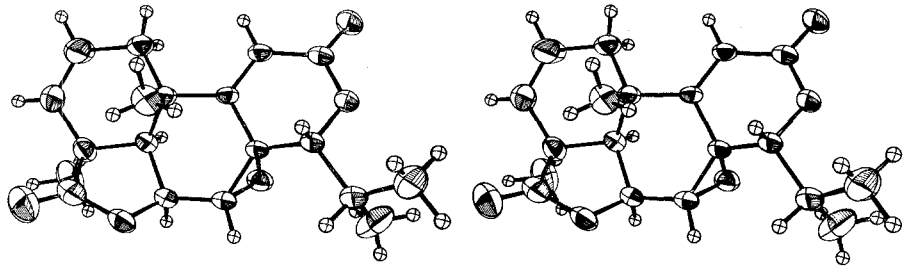


(**1**)



(**2**) R = H
(**3**) R = $COCH_3$

Crystals of (**1**) are monoclinic, space group $P2_1$, with a = 12.165 (3), b = 7.771 (1), c = 9.708 (4) Å, β = 113.76 (3)°, and Z = 2. The structure was solved by direct methods of phase determination (MULTAN[7]) and refined by block-diagonal least-squares techniques to yield R = 0.047 for 1975 independent reflections whose intensities were measured by counter diffractometry with monochromatic Mo-K_α radiation. Anisotropic thermal parameters were assumed for the non-hydrogen atoms, and all hydrogen atoms were located and included in the refinement.



Stereoscopic view of the molecular structure of (**1**). Thermal ellipsoids (ORTEP II) for the non-hydrogen atoms are drawn at the 50% probability level.

[1] Tumor Inhibitors. 99. For previous paper in the series, see S. M. KUPCHAN, Recent Adv. Phytochem., in press.
[2] For a recent review see M. N. GALBRAITH, D. H. S. HORN, S. ITÔ, M. KODAMA and J. M. SASSE, Agric. Biol. Chem. *36*, 2393 (1972).
[3] Tumor-inhibitory activity and cytotoxicity were assayed under the auspices of the National Cancer Institute, by the procedures described by R. I. GERAN, N. H. GREENBERG, M. M. MCDONALD, A. M. SCHUMACHER and B. J. ABBOTT (Cancer Chemother. Rep. part 3, *3*, 1 (1972)). Podolide showed significant activity in vivo against P-388 leukemia in mice and cytotoxicity in vitro towards cells derived from both human carcinoma of the nasopharynx (KB) and P-388 murine leukemia.
[4] Podolide was accompanied by trace amounts of its 2,3-dihydro derivative which could be removed by fractional crystallization from methanol.
[5] S. ITÔ, M. KODAMA, M. SUNAGAWA, T. TAKAHASHI, H. IMAMURA and O. HONDA, Tetrahedron Lett. *1968*, 2065.
[6] The diol (**2**) was characterized as its diacetate (**3**), $C_{23}H_{30}O_7$, mp 144–145°.
[7] G. GERMAIN, P. MAIN and M. M. WOOLFSON, Acta cryst. A *27*, 368 (1971).
[8] R. BUCOURT and D. HAINAUT, Bull. Soc. Chim. Fr. *1966*, 501.
[9] S. ITÔ, M. KODAMA, M. SUNAGAWA, M. KOREEDA and K. NAKANISHI, Chem. Commun. *1971*, 855.
[10] S. M. KUPCHAN and R. M. SCHUBERT, Science *185*, 791 (1974).
[11] We thank Dr. R. E. PERDUE, JR., U.S.D.A., for supplying the plant material (collected in Ethiopia in November, 1964). The work was supported by grants from the National Cancer Institute (CA-11718 and CA-11760) and the American Cancer Society (CI-102J), and a contract with the Division of Cancer Treatment, National Cancer Institute (NO1-CM-12099).

A stereoscopic representation of the molecular structure of (**1**) is shown in the Figure. The double bond in ring *A* is identified as C (2)–C (3). The lactone ring is a C (5) envelope, the cyclohexene and cyclohexane rings have slightly distorted 1,2-diplanar (*sofa*) conformations, while the pyran ring has a slightly distorted 1,3-diplanar conformation[8].

The absolute configuration of podolide (**1**) was assigned on the basis of the observation of a negative Cotton effect in the CD spectrum, $[\Phi]_{262}^{MeOH} - 15{,}090$[5,9]. Studies are in progress to determine the relative importance of the α,β-unsaturated δ-lactone, epoxide[10], and other functions with respect to the tumor-inhibitory activity of podolide[11].

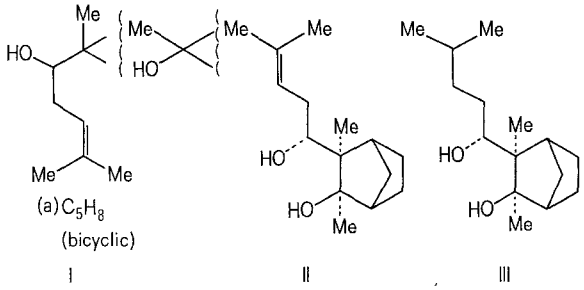
Zusammenfassung. Nachweis, dass Podolid, ein neues antileukämisches norditerpinisches Dilacton aus *Podocarpus gracilior* Pilg. die Struktur (**1**) besitzt.

S. M. KUPCHAN, R. L. BAXTER, MYRA F. ZIEGLER, P. M. SMITH and R. F. BRYAN[11]

Department of Chemistry, University of Virgina, Charlottesville (Virgina 22901, USA), 6 November 1974.

The Structure of Clausantalene, a New Sesquiterpene from *Clausena indica* Oliv.[1]

From the roots of *Clausena indica* Oliv. (Rutaceae) some known coumarins[2], a new cyclopropyl coumarin[3] and two new carbazole alkaloids[4,5] have been isolated. In this communication we report isolation of a new sesquiterpene designated clausantalene and its structure determination based on spectral and X-ray crystallographic data. Hexane extract of the roots, on careful chromatographic separation on silica gel and preparative TLC, gave about 0.01% yield of colourless crystals of clausantalene $C_{15}H_{26}O_2$, m.p. 114°, $[\alpha]_D + 27.7°$ (C 1.9, $CHCl_3$) (Rf 0.55, $CHCl_3 - 2\%$ MeOH; TLC Si gel). It has no UV-absorption and its IR-spectrum indicated the presence of hydroxyl groups (3380 cm^{-1}). Its mass spectrum showed a negligible molecular ion peak at *m/e* 238 and a base peak at *m/e* 220 ($M^+ - H_2O$). The other major fragment ions at *m/e* 177 ($220 - C_3H_7$), 151 ($220 - C_5H_9$) suggested the presence of an isopentenyl chain. This was confirmed by its NMR spectrum (100 MHz, $CDCl_3$) which showed gem-dimethyls at δ 1.65 and 1.72 (3H each, J = 1 Hz), and a slightly split triplet at δ 5.25 (1H, J = 7 Hz) assigned to an olefinic proton adjacent to an unsubstituted methylene group appearing at δ 2.1 (confirmed by decoupling). Irradiation at δ 1.72 sharpened the triplet at δ 5.25. A one-proton triplet at 3.88 (J = 7 Hz), also coupled to the methylene protons at δ 2.1 as shown by double resonance experiments, indicated that the proton should be placed on a methylene carbon having an oxygen function. 2 broad signals (1H each) at δ 2.7 and 3.3 vanished on deuteration, indicating the presence of 2 hydroxyl groups. 2 tertiary methyls appeared at δ 0.9 and 1.2, of which the latter should be due to a methyl on a carbon bearing a hydroxyl group. Hydrogenation over Pd-C or PtO_2 afforded dihydroclausantalene $C_{15}H_{28}O_2$, m.p. 102° $[\alpha]_D + 16.6°$ (C 2, $CHCl_3$) by reduction of the isopentenyl double bond. Since the dihydroderivative does not contain any unsaturation (NMR; no tetranitromethane colour), clausantalene should be a bicyclic sesquiterpene containing the 5-carbon chain (a). A partial structure (I) could be written on the evidence cited above and clausantalene should therefore belong to the sesquicarane, bergamotane or β-santalane types[6].



I II III

[1] Contribution No. 377 from Ciba-Geigy Research Centre.
[2] B. S. JOSHI, V. N. KAMAT and D. H. GAWAD, Phytochemistry *10*, 480 (1971).
[3] B. S. JOSHI, V. N. KAMAT and D. H. GAWAD, Experientia *30*, 223 (1974).
[4] B. S. JOSHI and D. H. GAWAD, Indian J. Chem. *10*, 1123 (1972).
[5] B. S. JOSHI and D. H. GAWAD, Indian J. Chem. *12*, 437 (1974).
[6] T. K. DEVON and A. I. SCOTT, *Handbook of Naturally Occurring Compounds* (Academic Press, New York 1972), Vol. 2, p. 55.