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PODOBLASTIN A, B AND C. NEW ANTIFUNGAL 3-ACYL-4-HYDROXY-5,6-DIHYDRO-2-PYRONES OBTAINED FROM *PODOPHYLLUM PELTATUM* L[1)]

Masakazu MIYAKADO*, Satoru INOUE, Yoo TANABE, Keisuke WATANABE,
Nobuo OHNO and Hirosuke YOSHIOKA
Pesticide Research Department, Sumitomo Chemical Co. Ltd.,
4-2-1 Takatsukasa, Takarazuka, Hyogo 665
Tom J. MABRY
The Department of Botany, The University of Texas at Austin,
Austin, TX 78712, U.S.A.

The potent antifungal constituent against *Pyricularia oryzae* Cav. obtained from *Podophyllum peltatum* L. has been disclosed to be a mixture of three new 3-acyl-4-hydroxy-5,6-dihydropyrones (podiblastin A, B and C). The absolute configuration at C-6 of podoblastins was determined to be (R).

In connection with our continuing screening program in search of pesticidally active substances of natural origin, we obtained from *Podophyllum peltatum* L. (Fam. Berberidaceae, May Apple)[2)], a well known medicinal plant[3)] in northwestern U.S.A., a mixture of 3-acyl-4-hydroxy-5,6-dihydro-2-pyrones which showed remarkable antifungal activity against *Pyricularia oryzae* Cav. (rice blast, see Table 1). The present communication describes structure elucidation of the new dihydropyrones designated as podoblastin A, B and C.

The $CHCl_3$ extract of aerial part of *P. peltatum* was separated by repeated column and preparative-layer chromatography (SiO_2) with monitoring of fractions for antifungal activity, giving crystalline active fraction, m.p. 45.0 - 46.5 ^{o}C[4)], in 0.40% yield. Although the active fraction was homogeneous on TLC, HPLC and GLC analyses, its ^{1}H- and ^{13}C-NMR spectra indicated that it was a mixture of structurally related analogues. The separation of these analogues was tried extensively by utilizing GLC (capillary columns) and HPLC (normal and reverse phase columns) but all the efforts were not successful. The presence of 3-acyl-4-hydroxy-5,6-dihydro-2-pyrone moiety was indicated by the close similarity between UV spectra of the active fraction (λmax 274 and 217 nm) and that of 3-acetyl-4-hydroxy-6-methyl-5,6-dihydro-2-pyrone (λmax 271 and 216 nm).[5,6)] A characteristically low field singlet at δ 17.9 in the ^{1}H-NMR was also consistent with the chromophore.[5)] On the basis of the ^{1}H- and ^{13}C-NMR spectral data assisted by ^{1}H-^{1}H decoupling experiments (see Figure 1), partial structure A was proposed for the active principles. It gave three ions of significant intensities in the mass spectrum at m/z 366.277 (366.276 for $C_{22}H_{38}O_4$), m/z 338.247 (338.243 for $C_{20}H_{34}O_4$) and m/z 336.232 (336.230 for $C_{20}H_{32}O_4$).

These three ions were proved to be the parent ions corresponding to three related components of the active fraction when a series of degradation reactions and subsequent GLC analysis[7] of the methylated products were conducted as shown in Figure 2.

The GLC analysis indicated the presence of three related methyl esters IVa, IVb and IVc in a ratio of 32 : 50 : 18 respectively, and a methyl ester of hydroxycarboxylic acid V. GLC-MS analysis of the former three methyl esters confirmed the structures of IVa, IVb and IVc as indicated in Figure 2, the above GLC ratio representing the relative abundance of Ia, Ib and Ic in the active fraction. The methyl ester V was identified to be methyl 3-hydroxyhexanoate by comparison with authentic specimen.[8]

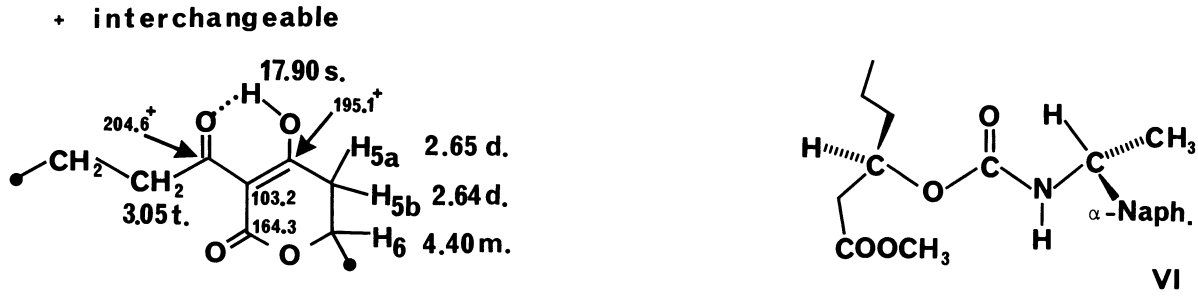
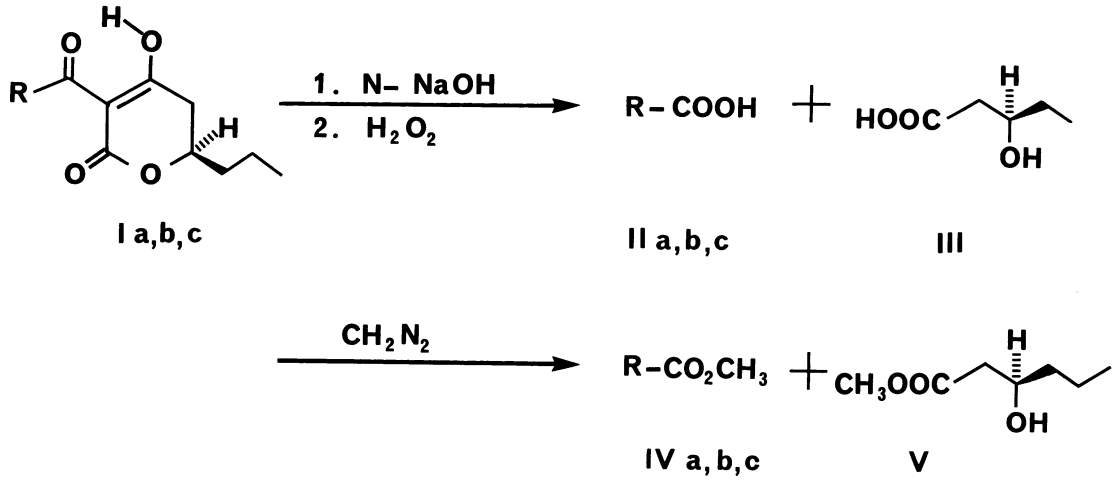


Figure 1. Partial structure A



a: R = $CH_3(CH_2)_{10}$— Podoblastin A

b: R = CH_2=CH-$(CH_2)_9$— Podoblastin B

c: R = $CH_3(CH_2)_{12}$— Podoblastin C

Figure 2.

The presence of the hydroxy ester V in the degradation products wolud be most reasonably elucidated by introducing a propyl group on C-6 of the partial structure A. The absolute configuration of the hydroxy ester V has been determined

according to the Parkle's method[9] as follows. The two diastereomeric carbamates that were derived from (R)-(-)-1-naphthylethyl isocyanate and synthetic (+)-methyl 3-hydroxyhexanoate were resolved well on HPLC analysis (μ-porasil, 4 mm x 25 cm, n-hexane-EtOAc 9:1 v/v, UV 280nm detection). The early emerging peak of the HPLC analysis was identified to be N-(R)-1-naphthylethyl-O-(R)-3-(1-methoxycarbonyl)-pentyl carbamate VI, since it gave on silanolysis[10] (-)-hydroxy acid and the absolute configuration of the (+)-enantiomer had been determined to be (S) by R. U. Lemieux et al.[11] The carbamate derived similarly from the natural hydroxy ester V gave a single peak on HPLC analysis which corresponded to the early emergent peak.[12] Therefore, the absolute configuration of V could be assigned to be (R) as shown in Figure 2. In conclusion, the structures of podoblastin A, B and C are rationally proposed as shown by Ia, Ib and Ic. Although podoblastins are structurally related to a phytotoxic fungal metabolite, alternalic acid, isolated from Alternalia solani[6], our result reprents the first isolation of this type of compounds from higher plants. The antifungal potency of podoblastin B Ib against P. oryzae is remarkably high for a natural product (Table 1). The proposed structures have been unambiguously confirmed by the total synthesis of each component, podoblastin A, B and C which is the subject of succeeding communication.[13]

Table 1. Antifungal activities of P. peltatum constituents against P. oryzae (rice blast disease, pot test)[14].

Materials	Disease control (%)[a]				
	100	50	25	12.5	6.3 (μg/ml)
$CHCl_3$ crude extract	52	31	0	0	0
podoblastin A, B and C[b]	84	69	37	11	0
podoblastin A (Ia)[c]	6	0	0	0	0
podoblastin B (Ib)[c]	85	85	31	8	0
podoblastin C (Ic)[c]	31	0	0	0	0

a) after 4 days incubation, disease severity was determined by the percentage of infected leaf area.
b) natural specimen (composed of podoblastin A : B : C = 32 : 50 : 18).
c) synthetic specimen (racemic compound, see succeeding communication[13]).

Acknowledgements

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References

1) Part 1 in the series of "Studies on Podoblastin."

2) Plant material was collected in Crockett National Forest, Texas, U.S.A. in April, 1979.

3) a) J.L. Hartwell and A.W. Schrecker, "The Chemistry of Podophyllum", Fortschr. Chem. Org. Naturst., 15 83-166 (1958).
b) The Merck Index, (9th Ed.), p.981 (1976).
c) P.M. Dewick and D.E. Jackson, Phytochemistry, 20 2277-2280 (1981).

4) R_f 0.60 (SiO_2 TLC, CH_2Cl_2). $[\alpha]_{22^\circ}^{\lambda}$

589	577	546	435	365nm
-25.3°	-28.1°	-38.0°	-84.4°	-199.8°

(c. 0.71, $CHCl_3$).
$E_{1cm}^{1\%}$ (EtOH), 305 (274 nm) and 195 (217 nm). IR ($CHCl_3$): 2910, 2840, 1710, 1540, 1455, 1210, 1060 and 910 cm^{-1}. ^{13}C-NMR (25.0 MHz, $CDCl_3$): δ 204.6 (s), 195.1 (s), 164.3 (s), 139.2 (d), 114.1 (t), 103.2 (s), 73.6 (d), 38.5 (t), 38.0 (t), 36.7 (t), 33.8 (t), 31.9 (t), 29.7 (methylene carbons), 29.0 (t), 25.1 (t), 22.7 (t), 18.0 (t), 14.1 (q) and 13.7 (q). ^1H-NMR (90.0 MHz, $CDCl_3$): δ 17.9 (s. 1H), 4.40 (m. 2H), 3.04 (t. J = 8.0 Hz, 2H), 2.65 (d. J = 6.0 Hz, 1H), 2.64 (d. J = 5.0 Hz, 1H), 1.26 (br.s. about 22H), 0.96 (t. J = 8.0 Hz, 3H), relatively low intensity signals at δ 5.80 (m. ca 0.5Hxl), 5.00 (m. ca 0.5Hx2) for terminal olefinic protons.

5) S. Gelin and R. Gelin, Bull. Soc. Chim. Fr., 288 (1968).

6) B. Keith, J. Chem. Soc., 860, 1662 (1960).

7) GLC conditions: t_R for IVa : IVb : IVc = 23.6 : 25.5 : 27.9 min. (2% DGSP 2m, oven temp. 100 - 200 °C (temp. programmed analysis, 5 °C/min.). t_R for V 4.0 min. (5% OV-17 1m, oven temp. 80 °C).

8) Methyl 3-hydroxyhexanoate V was synthesized from butylaldehyde and methyl bromoacetate by Reformatsky reaction.

9) W.H. Parkle and P.E. Adams, J. Org. Chem., 43 378 (1978).

10) W.H. Parkle and M.S. Hoekstra, J. Org. Chem., 39 3904 (1974).

11) R.U. Lemieux and J. Giguere, Can. J. Chem., 29 678 (1951).

12) The early emergent peak from 10 runs of the HPLC analysis were collected and the eluate was concentrated to give the carbamate VI (22 mg, isomer ratio 80.0 : 20.0, $[\alpha]_D$ +10.1°). The carbamate VI was silanolized to give (-)-3-hydroxy ester V (7 mg, $[\alpha]_D$ -16.8° ($CHCl_3$), reported value for (S)-isomer $[\alpha]_D$ +28.0° ($CHCl_3$)).

13) Y. Tanabe, M. Miyakado, N. Ohno and H. Yoshioka, Chem. Lett., 1982, 1543.

14) A definite amount of the testing sample (emulisified with Sorpol® and water) were sprayed on rice plant (Oryza sativa L. var. Kinki No. 33, 2.5th leaf stage). After 4 hrs, inoculation of P. oryzae was carried out by spraying a spore suspension contained about 10^7 spores/ml to the plant, then incubated at 28°C (humidity, 95% over) for 4 days.

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