

NEW CONGENERS OF CYTOTOXIC NOR-DITERPENOID DILACTONES IN PODOCARPUS NAGI:
THREE NEW COMPONENTS OF 7,8-EPOXY-ENOLIDE TYPE

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In the course of the thorough investigation in cytotoxic components of Podocarpus nagi Zoll. et Moritzi, we have recently reported several nor- and bisnor-diterpenoid dilactones with α -pyrone ring as active minor components in seed endosperms¹⁾. This paper reports three new members, (1), (2) and (3), of 7,8-epoxy-9,11-enolide type, which were isolated from fresh root bark, another abundant source of the active dilactones. Unlike the seed principles, the active components in the root bark generally remain at lower oxidation stages. The compounds, 1 and 2, co-occur as a 1:1 mixture (pmr) at slightly lower Rf region (SiO₂ plate) than nagilactone E (5)²⁾, a major component of the root bark. Both compounds show very similar chromatographic behaviors. Usual separation method, TLC or HLC, does not distinguish them by any of the single operation. Only a combined technique of the adsorption chromatography with droplet counter-current chromatography³⁾ gave a satisfactory result, and 1 and 2 were isolated in pure crystalline forms.

The compound (1): mp 225°, C₁₉H₂₂O₆, $\lambda_{\max}^{\text{MeOH}}$ 218 nm (ϵ :8900), ν_{\max}^{KBr} 3540, 1775, 1700 cm⁻¹, m/e(20 eV) 346.1442(M⁺, 19), 316.1337(M⁺-CH₂O, 19), 289.1096(M⁺-C₃H₅O, 100), 271.1311(M⁺-C₂H₃O₃, 16), 225.1056(22), 211.0921(16), 199.0882(17), $[\theta]_{262\text{nm}}^{\text{MeOH}}$ -21000. A characteristic of 7 α ,8 α -epoxy-9,11-enolide system is presented by pmr parameters of B/C ring protons, H-5 α , H-6 α , H-7 β , H-11 and

H-14 β , which can be correlated to those of nagilactone E (5) (Table 1). The sole hydroxyl group is placed at C-16⁶) on the following spectral bases: (i) One doublet signal of methyl protons in 5 is replaced by two-proton signals (AB part of an ABX system) of carbinyll hydrogens (H-16) at 4.08 and 4.30 ppm, which displace to 4.24 and 4.81 ppm, respectively, on acetylation. (ii) The corresponding methyl carbon (21.3 ppm) in 5 is replaced by a primary carbinyll carbon (62.3 ppm) in 1. In addition, some down-field shift ($\Delta\delta$: 7.8 ppm) of C-15 signal from 5 (26.7 ppm) to 1 (34.5 ppm) would reflect an effect of the hydroxyl group at C-16¹¹). The second olefinic bond is located on ring A from the next reasons: Two olefinic carbons gave doublet signals centered at 126.4 and 128.3 ppm, respectively, on a off-resonance measurement, and the corresponding olefinic proton signals appear in a modified AB type at 5.88 and 5.98 ppm. Saturation of an allylic methylene proton (2.06 ppm, H-1 β) resulted in about 20% area increase (NOE) of the H-11 signal, which establishes the double bond to be at 2,3-position. The pmr parameters of H-1, H-2 and H-3 are also compatible with those of podolactone D⁴) (6). Thus, 16-hydroxy-podolide is proposed for the structure of the compound (1).

The compound (2), mp 268°, C₁₉H₂₄O₆, $\lambda_{\max}^{\text{MeOH}}$ 217 nm (ϵ :11400), ν_{\max}^{KBr} 3440, 1773, 1706 cm⁻¹, m/e(20 eV) 348(M⁺, 4), 318(27), 291(100), 289(41), 261(41), 215(16), 201(11), $[\theta]_{262\text{nm}}^{\text{MeOH}}$ -24900. The pmr spectrum of 2 almost overlaps with that of 1, except for the absence of the olefinic proton signals at C-2/C-3 (Table 1). Therefore, 2,3-dihydro-16-hydroxy-podolide is given as the structure of 2. Stereochemistry at C-15 in 1 and 2 remains undetermined.

The compound (3), mp 227° (sublime), C₁₉H₂₂O₆, $\lambda_{\max}^{\text{MeOH}}$ 217 nm (ϵ :10200), ν_{\max}^{KBr} 1780, 1712 cm⁻¹, m/e(20 eV) 346(M⁺, 2), 317(6), 303(73), 287(23), 285(23), 275(46), 257(35), 201(42), $[\theta]_{261\text{nm}}^{\text{MeOH}}$ -21400, shows an R_f value higher than nagilactone E (5). The carbinyll proton signals at 3.27 and 3.39 ppm reveal the presence of an additional epoxide group, which should be placed at 2 β ,3 β -position⁵) by the pmr comparisons with sellowin B^{4b,6a}) and podolactone, A⁷) or C^{4a}). Two pairs of doublet methyl signals on pmr indicate the presence of an iso-propyl group at C-14. Therefore, 2 β ,3 β -epoxy-podolide is proposed for the structure of 3.

With the three new compounds, another crystalline substance (4), mp 248°, $C_{19}H_{24}O_5$, $\lambda_{\text{max}}^{\text{MeOH}}$ 218 nm ($\epsilon:10400$), $\nu_{\text{max}}^{\text{KBr}}$ 1775, 1705 cm^{-1} , $m/e(20 \text{ eV})$ 332(M^+ , 2), 304(2), 289(12), 273(14), 261(100), 245(63), 233(41), 217(32), $[\theta]_{262\text{nm}}^{\text{MeOH}} -24200$, was obtained from the least polar fraction. The spectral data are identical with the reported values of " Substance A " by Watts⁸⁾, which corresponds to 7 α , 8 α -epoxy-nagilactone F (or 2,3-dihydro-podolide⁹⁾).

The absolute configuration of the compounds, 1~4, is the same to that of nagilactone E²⁾, accordingly, of inumakilactone A¹⁰⁾, since the same sign of Cotton effect was obtained by cd measurements.

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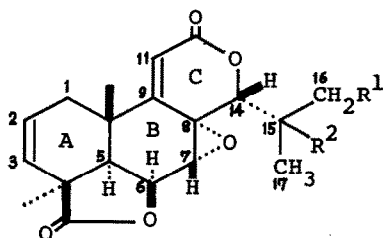
Table 1. The pmr parameters of the lactones (pyridine- d_5)

Lactones	H ¹	H ²	H ³	H ⁵	H ⁶	H ⁷	H ¹¹	H ¹⁴	H ¹⁶	H ¹⁷	CH ₃ [*]
(<u>1</u>)	2.06 br d	5.88 dt (10.0, 3.0, 3.0)	5.98 d (10.0)	2.10 d (5.5)	5.22 dd (5.5, 1.5)	4.40 d (1.5)	6.24 s	4.94 d (5.5)	**	1.34 d (7.0)	1.19 1.32
(<u>2</u>)	--	--	--	1.83 d (5.5)	5.14 dd (5.5, 1.5)	4.36 d (1.5)	6.19 s	4.88 d (5.5)	**	1.33 d (7.0)	1.14 1.17
(<u>3</u>)	***	3.39 m	3.27 d (4.0)	1.82 d (4.5)	5.15 dd (4.5, 1.5)	4.23 d (1.5)	6.16 s	4.54 d (4.0)	1.02 d (7.5)	1.16 d (7.5)	1.37 1.44
(<u>4</u>)	--	--	--	1.80 d (4.5)	5.07 dd (4.5, 1.5)	4.19 d (1.5)	6.13 s	4.54 d (4.5)	1.03 d (7.0)	1.17 d (7.0)	1.09 1.18
(<u>5</u>)	--	--	3.82 dd (6.0, 10.0)	1.90 d (4.5)	5.11 dd (4.5, 1.5)	4.23 d (1.5)	6.18 s	4.57 d (4.0)	1.03 d (7.0)	1.18 d (7.0)	1.31 1.55

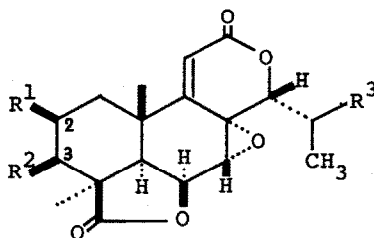
* singlet methyl signals. ** H¹⁶: 4.30 dd (4.5, 10.5), 4.08 dd (7.5, 10.5).

*** H^{1 α} : 1.73 dd (1.5, 14.5), H^{1 β} : 2.23 dd (2.0, 14.5).

s: singlet, d: doublet, dd: double doublet, dt: double triplet, m: multiplet
br: broad.



- (1) $R^1 = \text{OH}$, $R^2 = \text{H}$
 (6) $R^1 = \text{SOCH}_3$, $R^2 = \text{OH}$
 (7) $R^1 = R^2 = \text{H}$



- (2) $R^1 = R^2 = \text{H}$, $R^3 = \text{CH}_2\text{OH}$
 (3) $R^1, R^2 = >\text{O}$, $R^3 = \text{CH}_3$
 (4) $R^1 = R^2 = \text{H}$, $R^3 = \text{CH}_3$
 (5) $R^1 = \text{H}$, $R^2 = \text{OH}$, $R^3 = \text{CH}_3$

Notes and References

- Y. Hayashi, Y. Yuki, T. Matsumoto, T. Sakan, *Tetrahedron Lett.*, **1977**, 2953.
- Y. Hayashi, J. Yokoi, Y. Watanabe, T. Sakan, Y. Masuda, R. Yamamoto, *Chem. Lett.*, **1972**, 759.
- T. Tanimura, J. J. Pisano, Y. Ito, R. T. Bowman, *Science*, **169**, 54 (1970); 300 tubes ($\phi 2 \text{ mm} \times 400 \text{ mm}$), $\text{CHCl}_3:\text{MeOH}:\text{H}_2\text{O}$ (35:65:40) as solvent system.
- The ring A double bond of podolactone D is placed at 2,3- rather than 1,2- position: (a) M. N. Galbraith, D. H. S. Horn, J. Sasse, *Chem. Commun.*, **1971**, 1362. (b) S. K. Arora, R. B. Bates, P. C. C. Chou, W. E. Sanchez L., K. S. Brown, Jr., M. N. Galbraith, *J. Org. Chem.*, **41**, 2458 (1976).
- The ring A epoxide group of sellowin B and podolactone, A or C, is located at 2 β ,3 β -position^{4,6a,7}. The corresponding 2,3 α -epoxides ($J_{1\alpha,2\beta}$: 1.5, $J_{1\beta,2\beta}$: 6.0~6.5, $J_{2\beta,3\beta}$: 3.5~4.0 Hz) were prepared from podolide (7) and 16-hydroxy-podolide (1) by m-chloro-perbenzoic acid oxidation. The pmr parameters of H-1, H-2 and H-3 readily discriminate both orientations. Podolide (7) was derived from nagilactone E by dehydration with POCl_3 in pyridine. The details of these results will be published elsewhere.
- (a) W. E. Sanchez L., K. S. Brown, Jr., T. Nishida, L. J. Durham, A. M. Duffield, *An. Acad. Bras. Cienc.*, **42**, 77 (1970). (b) T. Hayashi, H. Kakisawa, S. Ito, Y. P. Chen, H. Y. Hsu, *Tetrahedron Lett.*, **1972**, 3385.
- M. N. Galbraith, D. H. S. Horn, J. M. Sasse, D. Adamson, *Chem. Commun.*, **1970**, 170. B. J. Poppleton, *Cryst. Struct. Commun.*, **4**, 101 (1975).
- D. J. Watts, "The Chemical Constituents of two *Podocarpus* species", Dissertation (1969), Indiana university.
- The compound (4) was identified by ir comparison with 2,3-dihydropodolide, which is accompanied by podolide in the original plant: S. M. Kupchan, R. L. Baxter, M. F. Ziegler, P. M. Smith, R. F. Bryan, *Experientia*, **31**, 137 (1975).
- S. Ito, M. Kodama, M. Sunagawa, T. Takahashi, H. Imamura, O. Honda, *Tetrahedron Lett.*, **1968**, 2065.
- J. B. Stothers, "Carbon-13 NMR Spectroscopy" Academic Press, N.Y., (1972) p139.