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NEW CONGENERS OF CYTOTOXIC NOR-DITERPENOID DILACTONES IN PODOCARPUS NAGI: THREE HIGHLY POLAR COMPONENTS WITH α-PYRONE RING

Yuji Hayashi*, Yo-ichi Yūki and Takeshi Matsumoto
Department of Chemistry, Faculty of Science, Osaka City University
Sugimotocho, Sumiyoshiku, Osaka 558, Japan

Takeo Sakan
The Institute of Food Chemistry
Shimamotocho, Mishimagun, Osaka 618, Japan

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Seeds and root bark of Podocarpus nagi are plentiful sources of biologically active nor- and bisnor-diterpenoid dilactones. In connection with the studies on the cytotoxicity[1,2] and the plant growth inhibitory activity[3] of nagilactones, active minor components of the plant have thoroughly been investigated for a crude extract. This paper reports the structures of three new members of the dilactones separated in small yield from highly polar solid fraction of the seed extract. Some difficulties have been experienced for the isolation of the components, mainly because of their poor solubility for common organic solvents. A combined technique of preparative TLC (multi-development) or column chromatography (SiO_2-chloroform) with droplet counter-current chromatography[9] was successful; the last method will be promising for separation of this type of compounds.

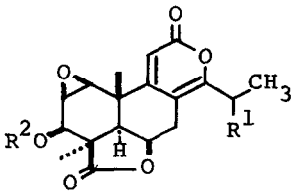
All of the three components, (1)∼(3),[4] have an α-pyrone partial structure in ring C (λ_{max} 300 nm) and the gross structures were assigned by spectral correlation with the known nagilactones, B (7) and D (8)[5].

Compound (1). $C_{18}H_{20}O_7$; λ_{max}^{EtOH} 225, 302 nm, ν_{max}^{KBr} 3400, 1740, 1690, 1610, 1530 cm^{-1}. Diacetate (4) (Ac_2O in pyridine), mp 273°, $\nu_{max}^{CHCl_3}$ 1770, 1730, 1705, 1630, 1550 cm^{-1}, m/e(70 eV) 432(M^+), 390, 389, 372, 345, 330, 303, 286, 271, 257. [Found, C 60.88, H 5.64%, Calc. for $C_{22}H_{24}O_9$, C 61.10, H 5.59%].

Pmr spectrum of 1 is closely similar to that of nagilactone D (8) (Table 1), except for the signals due to H-7α and C-14 side chain protons. A one-proton quartet at 5.18 ppm was assigned as a carbinyl hydrogen (6.11 ppm in diacetate), which couples with a doublet methyl signal at 1.68 ppm. From these facts, 15-hydroxy-nagilactone D is proposed for the structure of 1. The stereochemistry at C-15 remains unclear.

Compound (2). $C_{19}H_{24}O_7$, mp 247-9° (dec), λ_{max}^{EtOH} 217, 299 nm, ν_{max}^{KBr} 3450, 1780, 1660, 1610, 1530 cm^{-1}, m/e(70 eV) 364(M^+, 83), 346(28), 336(44), 321(42), 318(30), 303(42), 275(28), 259(33), 257(33), 229(39), 203(100), 201(53). [Found, C 62.55, H 6.62%, Calc. for $C_{19}H_{24}O_7$, C 62.62, H 6.64%]. Triacetate (5) (Ac_2O in pyridine), mp 237-239° (dec), λ_{max}^{EtOH} 231, 300 nm, $\nu_{max}^{CHCl_3}$ 1790, 1750, 1720, 1630, 1550 cm^{-1}, m/e(70 eV) 490(M^+). [Found, C 61.23, H 6.14%, Calc. for $C_{25}H_{30}O_{10}$, C 61.21, H 6.17%]. The relationship of three protons, H-5α, H-6α and H-7α, was determined by pmdr studies. Two doublet methyl signals (1.26, 1.31 ppm) as well as a quintet at 3.48 ppm show the presence of a iso-propyl group at C-14. Remaining two hydroxyl groups are placed at 1β and 3β positions on the bases of the following reasons: (i) A significant high field shift (Δδ: 1.33 ppm) of H-11 signal in a conversion of 2 to the triacetate[5]. (ii) A two-proton multiplet at 2.5 ppm interacts with a complex multiplet around 4.0 ppm (pmdr); the latter is analyzed as two pairs of double doublet due to two carbinyl protons at 1α (axial) and 3α (axial)[7]. Fragmentation by 70 eV electron impact gave closely similar spectra of 2 (3β-hydroxy-nagilactone A) and nagilactone B (2β-hydroxy-nagilactone A)[8].

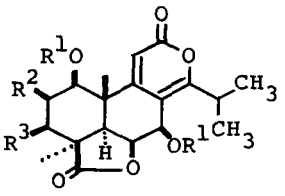
Compound (3), $C_{18}H_{22}O_7$, sublimes at 200-210°; λ_{max}^{EtOH} 224, 303 nm, ν_{max}^{KBr} 3400, 1750, 1700, 1620, 1550 cm^{-1}, m/e(70 eV) 350(M^+,100), 322(32), 304(58), 276(46), 258(53), 245(33), 229(33), 217(52), 203(47), 189(52), 187(84), 175(55), 161(82). Triacetate (6) (Ac_2O in pyridine), mp 283-5° (sublime), λ_{max}^{EtOH} 224, 306 nm, $\nu_{max}^{CHCl_3}$ 1790, 1760, 1720, 1640, 1560 cm^{-1}, m/e(70 eV) 476(M^+). [Found, C 60.30, H 5.93%, Calc. for $C_{24}H_{28}O_{10}$, C 60.50, H 5.92%]. The pmr signals of the functional protons on B/C ring are comparable in 3 with those of nagilactone D (8). Three hydroxyl groups are placed on ring A, at 1β, 2β and 3β, respectively, from the following reasons: (i) a large acetylation shift (Δδ: 1.28 ppm)



(1) R^1=OH, R^2=H

(4) R^1=$OCOCH_3$
R^2=$COCH_3$

(8) R^1=R^2=H

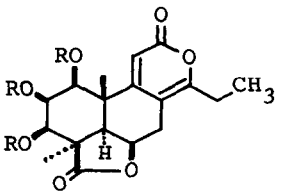


(2) R^1=R^2=H, R^3=OH

(5) R^1=$COCH_3$, R^2=H, R^3=$OCOCH_3$

(7) R^1=R^3=H, R^2=OH

(9) R^1=R^2= R^3=H



(3) R=H

(6) R=$COCH_3$

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

lactones	CH_3*	CH_3**	H^1	H^2	H^3	H^5	H^6	H^7	H^7	H^{11}	H^{15}
(1)	1.36 1.45	1.68 (6.5)	3.63 d (4.5)	3.57 dd (4.5, 6.0)	4.63 d (6.0)	1.88 d (7.0)	4.91 dt (6.0, 6.0, 10.0)	2.77 dd (6.0, 16.0)	3.88 dd (10.0, 16.0)	6.53 s	5.18 qua (6.5)
(4)	1.24 1.50	1.48 (6.5)	~3.7	~3.7	5.59 d (6.0)	2.15 d (6.5)	4.90 dt (6.5, 6.5, 9.5)	2.95 dd (6.5, 17.0)	3.67 dd (9.5, 17.0)	6.77 s	6.11 qua (7.0)
(2)	1.68 2.09	1.26 (7.0) 1.31 (7.0)	4.07 dd (5.0, 11.0)	2.5 m	3.95 dd (7.0, 12.0)	1.86 d (5.0)	5.25 dd (5.0, 9.0)	--	5.65 d (9.0)	7.39 s	3.48 qui (7.0)
(5)	1.43 1.79	1.09 (6.0) 1.15 (6.0)	5.3 ~5.5 m	2.3 ~2.7 m	5.3 ~5.5 m	2.22 d (5.0)	5.3 ~5.5 dd	--	6.65 d (9.5)	6.06 s	3.05 qui (6.0)
(3)	1.50 1.71	1.10 t (7.5)	3.94 d (7~8)	4.18 dd (7.0, 8.0)	3.94 d (7~8)	1.84 d (5.0)	5.10 dt (4.5, 5.0, 9.5)	2.90 dd (4.5, 17.0)	3.28 dd (9.5, 17.0)	7.24 s	2.44 qua (7.5)
(6)	1.54 1.54	1.08 t (8.0)	5.66 br,s	5.66 br,s	5.66 br,s	2.24 d (6.0)	5.14 dt (6.0, 7.0, 10.0)	2.89 dd (7.0, 17.0)	3.33 dd (10.0, 17.0)	5.96 s	2.42 qua (8.0)
(8)	1.44 1.51	1.10 t (7.5)	3.68 d (4.0)	3.52 dd (4.0, 6.0)	4.61 d (6.0)	1.88 d (6.0)	4.89 dt (6.0, 6.5, 10.0)	2.71 dd (6.5, 17.0)	3.34 dd (10.0, 17.0)	6.56*** s	2.47 qua (7.5)

* singlet methyl groups, ** doublet methyl groups unless otherwise specified,
*** 6.62 ppm in acetate (pyr-d_5).
s: singlet, d: doublet, t: triplet, dd: double doublet, dt: double triplet, qua: quartet, qui: quintet, m: multiplet, br: broad.

of H-11 signal[5] (a proof for 1β-hydroxyl group). (ii) A magnitude (7-8 Hz) of the coupling constants, $J_{1,2}$ and $J_{2,3}$, in 3 suggests the cis-relationships[7] of three protons, H-1, H-2 and H-3 [the corresponding protons are almost magnetically equivalent in acetate (6)]. (iii) The β-orientation is more probable for C-3 hydroxyl group because of their co-occurrence in the plant tissues with related 3β-hydroxy-lactones[3a,5b].

Absolute configurations of the three lactones should be the same as those of nagilactones, A (9) and B (7)[5c], as represented in the formula, since the same sign of Cotton effect was observed by CD measurements of the acetates[10].

Footnotes and References

1. Y. Hayashi, T. Sakan, Y. Sakurai, T. Tashiro, Gann, 66, 587 (1975).
2. Cytotoxicity of podolide, an analogous dilactone: S. M. Kupchan, R. L. Baxter, M. F. Ziegler, P. M. Smith, R. F. Bryan, Experientia, 31, 137 (1975).
3. a) Y. Hayashi, J. Yokoi, Y. Watanabe, T. Sakan, Chem. Letters, 1972, 759. b) Y. Hayashi, T. Sakan, Plant Growth Substances 1973: Proc. 8th Int. Conf. Plant Growth Subst.,p 525 (1974), and references cited therein.
4. Mobility on a TLC plate of the three new components decreases in this order. Rf values: nagilactone B (reference) 0.31, 1 0.22, 2 0.20, 3 0.09 [Silicagel G, $CHCl_3$:acetone(3:1), six-developments].
5. a) Y. Hayashi, S. Takahashi, H. Ona, T. Sakan, Tetrahedron Letters, 1968, 2071. b) S. Ito, M. Kodama, M. Sunagawa, H. Honma, Y. Hayashi, S. Takahashi, H. Ona, T. Sakan, T. Takahashi, Tetrahedron Letters, 1969, 2951. c) Y. Hayashi, T. Sakan, K. Hirotsu, A. Shimada, Chem. Letters, 1972, 349. The orientation of ring A substituents in nagilactones, C and D, should be revised to 1β,2β-epoxy-3β-hydroxy structure[6].
6. J. E. Godfrey, J. M. Waters, Aust. J. Chem., 28, 745 (1975); 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).
7. The ring A conformation of 2 and 3 is possibly in a chair form, since nagilactone B 2,7-bis-p-bromobenzoate exists in the same conformation (X-ray analysis: Y. Hayashi, T. Higuchi, unpublished result).
8. Fragmentation of nagilactone B (70 eV): m/e 364(M^+, 67), 346(13), 336(52), 321(87), 318(46), 303(85), 287(24), 285(21), 275(76), 257(50), 229(59), 203 (100), 201(41).
9. T. Tanimura, J. J. Pisano, Y. Ito, R. T. Bowman, Science, 169, 54 (1970); 300 tubes (φ2 mm × 400 mm), $CHCl_3$:MeOH:H_2O(35:65:40) as solvent system.
10. 4: $[\theta]_{293nm}$+24900, 5: $[\theta]_{295nm}$+3880, 6: $[\theta]_{300nm}$+4740.