Three New Terpenoids from *Pinus yunnanensis*

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A new abietatrienoid, 15-methoxydidehydroabietic acid (**1**), a new ring *B*-seco abietatrienoid, 10-hydroxy-9,10-secoabieta-8,11,13-trien-18-oic acid (**2**), and a new tetracyclic triterpenoid, pinusyunnanol (**3**), together with five known compounds, were obtained from the twigs and needles of *Pinus yunnanensis*. The structures of the new compounds were elucidated by means of extensive spectroscopic analyses.

Introduction. – The genus *Pinus* is known to be a rich resource of terpenoids [1–4] and fatty acids [5], some of which exhibited antimicrobial [6] and antiviral [7] activities. *Pinus yunnanensis* (Pinaceae) is an evergreen tall tree distributed in the southwest of China [8]. In our present work, three new compounds, 15-methoxydehydroabietic acid (**1**), 10-hydroxy-9,10-secoabieta-8,11,13-trien-18-oic acid (**2**), and pinusyunnanol (**3**), along with five known compounds, angustanoic acid D, 7,8-epoxy-1(12)-caryophyllen-9β-ol, junicedric acid, 12-hydroxydehydroabietic acid, and (24*S*)-3β-methoxy-5α-lanost-9(11)-ene-24,25-diol (**4**), were isolated from the EtOH extracts of the twigs and needles of *P. yunnanensis*. We report here the isolation and structure elucidation of these compounds by means of extensive spectroscopic analyses.

Results and Discussion. – Compound **1** was obtained as a colorless gum. The HR-ESI-MS displayed a *pseudo*-molecular-ion peak at *m*/*z* 353.2083 ($[M+Na]^+$), consistent with the molecular formula $C_{21}H_{30}O_3$ (calc. 353.2093 for $C_{21}H_{30}NaO_3^+$). The IR spectrum exhibited a CO group absorption band (1695 cm^{-1}), along with the typical broad band from 3500 to 2500 cm^{-1} pointing to a COOH group. The ^{1}H-NMR spectrum displayed signals of three aromatic H-atoms at δ(H) 7.21 (*d*, $J=8.0$), 7.15 (*d*, $J=8.0$), and 7.03 (*s*), representing a typical 1,3,4-trisubstituted benzene unit, of one MeO group at δ(H) 3.06 (*s*), and of four Me groups at δ(H) 1.50 (*s*, 6 H), 1.29 (*s*, 3 H), and 1.22 (*s*, 3 H). In accordance with the molecular formula, all 21 C-atoms were resolved in the ^{13}C-NMR spectrum and attributed by DEPT experiments to four Me and five sp^3-CH_2 groups, one sp^3-CH group, three quaternary sp^3-C-atoms, including one O-bearing at δ(C) 76.6, one MeO group at δ(C) 50.6, six aromatic C-atoms (including three sp^2-CH groups), and one COOH group at δ(C) 185.1 (*Table 1*). All the data mentioned above revealed a tricyclic abietatriene backbone for compound **1**. The HMBC and HSQC spectra confirmed this deduction and led to the constitutional formula **1**. The HMBCs

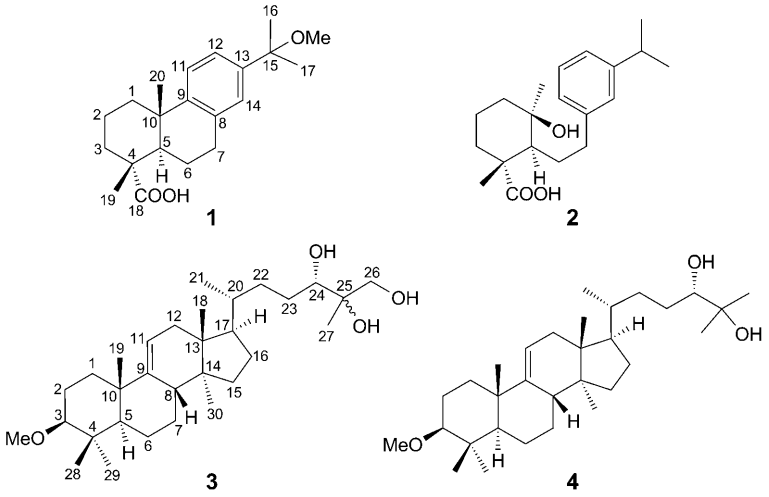

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**[a])

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.47–1.54 (*m*), 2.32 (*d*, *J* = 12.2)	37.8	1.48–1.54 (*m*), 1.68–1.70 (*m*)	40.4
CH_2(2)	1.74–1.80 (*m*, 2 H)	18.5	1.50–1.54 (*m*, 2 H)	17.5
CH_2(3)	1.69–1.76 (*m*), 1.76–1.84 (*m*)	36.6	1.69–1.74 (*m*), 1.78–1.82 (*m*)	37.2
C(4)	–	47.4	–	47.5
H–C(5)	2.25 (*d*, *J* = 12.3)	44.5	1.93–1.97 (*m*)	48.1
CH_2(6)	1.54–1.58 (*m*), 1.83–1.88 (*m*)	21.7	1.49–1.55 (*m*), 1.76–1.81 (*m*)	30.3
CH_2(7)	2.84–2.98 (*m*)	30.1	2.65–2.73 (*m*)	37.5
C(8)	–	134.6	–	142.6
C(9) or H–C(9)	–	147.8	6.97 (*d*, *J* = 7.5)	125.7
C(10)	–	36.9	–	72.3
H–C(11)	7.21 (*d*, *J* = 8.0)	124.0	7.16 (*dd*, *J* = 7.5, 7.5)	128.3
H–C(12)	7.15 (*d*, *J* = 8.0)	123.2	7.04 (*d*, *J* = 7.5)	123.7
C(13)	–	142.5	–	148.9
H–C(14)	7.03 (*s*)	126.3	7.00 (*s*)	126.5
C(15) or H–C(15)	–	76.6	2.83–2.90 (*m*)	34.1
Me(16)	1.50 (*s*)	27.9	1.24 (*d*, *J* = 6.9)	23.9
Me(17)	1.50 (*s*)	27.8	1.24 (*d*, *J* = 6.9)	24.0
C(18)	–	185.1	–	185.2
Me(19)	1.29 (*s*)	16.2	1.37 (*s*)	16.6
Me(20)	1.22 (*s*)	25.1	1.30 (*s*)	31.3
MeO	3.06 (*s*)	50.6		142.6

[a]) Recorded at 400 (^{1}H-NMR) and 100 (^{13}C-NMR) MHz in $CDCl_3$; δ in ppm, *J* in Hz.

of Me(19) to C(3), C(4), C(5), and C(18) placed the COOH group at C(4), and the HMBCs of Me(16) and Me(17) to C(15) and C(13), as well as the HMBCs of the MeO group at δ(H) 3.06 (*s*) to C(15) located the MeO group at C(15) (*Fig. 1*, *a*). The relative

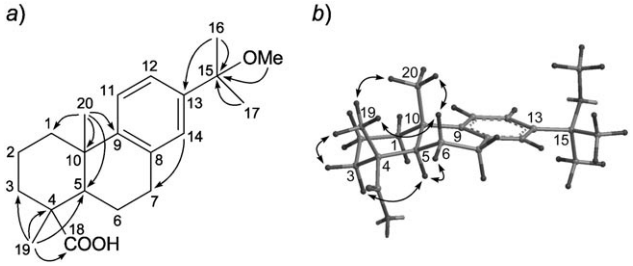

Fig. 1. a) *Selected HMBCs* (H → C) *and* b) *key ROESY correlations* (H ↔ H) *of* **1**

configuration of **1** was mainly deduced by the ROESY spectrum (*Fig. 1,b*). The ROESY correlations of Me(19)/Me(20), Me(19)/H_β–C(3), and Me(20)/H_β–C(6) showed that they were cofacial and were arbitrarily assigned β-orientation. Accordingly, the ROESY correlations of H–C(5)/H_α–C(3) and H–C(5)/H_α–C(6) indicated that they are on the same side and α-oriented. From these data, the structure of compound **1** was assigned as depicted.

Compound **2**, a colorless gum, was assigned the molecular formula $C_{20}H_{30}O_3$ based on HR-ESI-MS ($C_{20}H_{30}NaO_3^+$ at *m/z* 341.2102; calc. 341.2093) in combination with the NMR data, with six degrees of unsaturation. The IR spectrum showed the presence of OH (3423 cm^{-1}) and CO (1693 cm^{-1}) groups, together with the broad-band absorption from 3500 to 2500 cm^{-1}, typical for a COOH group. The ^{1}H-NMR spectrum displayed two Me *singlet*s at δ(H) 1.30 (*s*) and 1.37 (*s*), a *doublet* for two Me groups at δ(H) 1.24 (*d*, *J* = 6.9, 6 H), indicating the presence of a terminal ^{i}Pr group, and signals for four aromatic H-atoms at δ(H) 7.16 (*dd*, *J* = 7.5, 7.5), 7.04 (*d*, *J* = 7.5), 7.00 (*s*), and 6.97 (*d*, *J* = 7.5), representing a typical 1,3-disubstituted benzene ring. The ^{13}C-NMR and DEPT spectra displayed resonances for four Me, five CH_2, and two sp^3-CH groups, two quaternary sp^3-C-atoms, including one O-bearing at δ(C) 72.3, six aromatic C-atoms including four sp^2-CH groups, and one COOH group at δ(C) 185.2 (*Table 1*). The above functional groups accounted for five out of six degrees of unsaturation, the remaining one degree of unsaturation required one additional ring in compound **2** besides the benzene ring. Its ^{13}C-NMR data resembled those of **1** except for the absence of one sp^3 quaternary C-atom and one quaternary aromatic C-atom, and the presence of one more sp^3-CH C-atom and one more aromatic CH group. These spectral data implied that compound **2** was likely a *B*-seco abietatrienoid. Analysis of 1D- and 2D-NMR data further revealed that **2** possessed a feature of 9,10-secoabietatrienoid which is very rare in natural products. In the HMBC experiment, the correlations of Me(20) to C(1), C(5), and C(10) indicated that the OH group should be at C(10), and the correlations of Me(19) to C(3), C(4), C(5), and C(18) placed the COOH group at C(4) (*Fig. 2,a*). The ROESY experiment allowed the establishment of the relative configuration of **2** (*Fig. 2,b*), in which the Me(20) and H–C(5) were proved to be α-oriented, whereas Me(19) was in β-orientation. Thus, the structure of **2** was determined as depicted.

For compound **3**, a colorless solid, the molecular formula $C_{31}H_{54}O_4$ was determined by HR-EI-MS at *m/z* 490.4033 (M^+; calc. 490.4022) with five degrees of unsaturation. The IR spectrum showed the presence of OH groups (3408 cm^{-1}). The ^{13}C-NMR

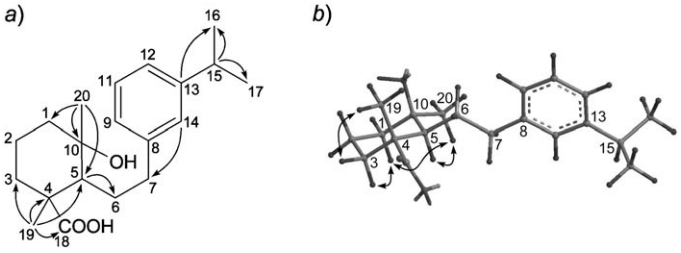

Fig. 2. a) *Selected HMBC* (H → C) *and* b) *key ROESY correlations* (H ↔ H) *of* **2**

including DEPT experiments showed that one of the five degrees of unsaturation came from a trisubstituted C=C bond (δ(C) 114.7 and 148.7). The remaining four degrees of unsaturation were, therefore, indicative of tetracyclic structure of **3**. In addition, the 1D-NMR data, along with the HSQC spectrum, showed the presence of seven Me goups (δ(H) 1.10 (*s*), 1.04 (*s*), 0.97 (*s*), 0.90 (*d*, $J = 6.6$), 0.80 (*s*), 0.74 (*s*), and 0.65 (*s*)), one O-bearing CH_2 group (δ(H) 3.83 (*d*, $J = 11.4$) and 3.47 (*d*, $J = 11.4$)), two O-bearing CH groups (δ(H) 3.43 (*d*, $J = 11.2$) and 2.65 (*dd*, $J = 11.4$, 4.1)), and one olefinic H-atom (δ(H) 5.22 (*d*, $J = 5.9$)) (*Table 2*). The above data implied that **3** was a tetracyclic triterpene. Careful analysis of 1D- and 2D-NMR data revealed that compound **3** was very similar to (24*S*)-3β-methoxy-5α-lanost-9(11)-ene-24,25-diol (**4**) [9], which was also isolated in this investigation, except for the replacement of a Me group by an O-bearing CH_2 group in **3**. The HMBCs of Me(27) to the three O-bearing C-atoms (C(24), C(25), and C(26) at δ(C) 79.3, 73.9, and 67.7) enabled us to locate the O-bearing CH_2 C-atom as C(26) (*Fig. 3*, *a*). Concerning the configuration, the ROESY spectrum and the almost identical ^{1}H-NMR spectra established that compound **3** possessed the same relative configuration as *rel*-(3β,24*S*)-3-methoxylanost-9(11)-ene-24,25,26-triol (**4**; *Fig. 3*, *b*) The relative configuration at C(25) remains to be determined.

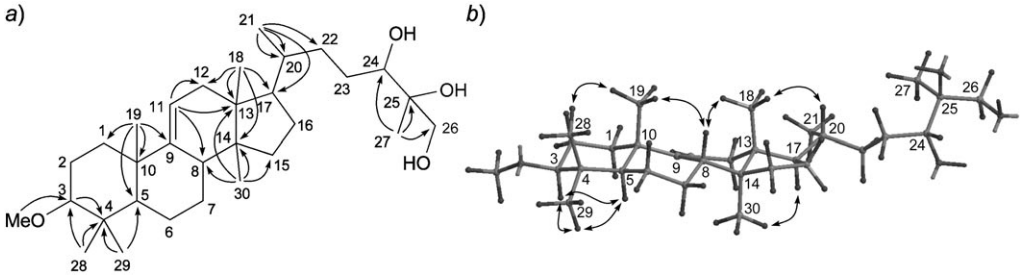

Fig. 3. a) *Selected HMBC* (H → C) *and* b) *key ROESY correlations* (H ↔ H) *of* **3**

Besides these three new compounds, five known compounds, angustanoic acid D [10], 7,8-epoxy-1(12)-caryophyllen-9β-ol [11], junicedric acid [12], 12-hydroxydehydroabietic acid [13], and (24*S*)-3β-methoxy-5α-lanost-9(11)-ene-24,25-diol [9] were isolated and identified by means of spectroscopic methods.

Table 2. *^{1}H- and ^{13}C-NMR Data of* **3**[a])

	δ(H)	δ(C)
CH_2(1)	1.36–1.40 (*m*), 1.79–1.86 (*m*)	36.0
CH_2(2)	1.26–1.38 (*m*), 1.84–1.94 (*m*)	28.0
H–C(3)	2.65 (*dd*, *J* = 11.4, 4.1)	88.6
C(4)	–	39.0
H–C(5)	0.82–0.90 (*m*)	53.0
CH_2(6)	0.85–0.88 (*m*), 1.88–1.94 (*m*)	22.5
CH_2(7)	1.02–1.06 (*m*), 1.62–1.70 (*m*)	21.2
C(8)	–	41.8
C(9)	–	148.7
C(10)	–	39.4
H–C(11)	5.22 (*d*, *J* = 5.9)	114.7
CH_2(12)	1.84–1.94 (*m*), 2.02–2.10 (*m*)	37.1
C(13)	–	44.3
C(14)	–	47.0
CH_2(15)	1.30–1.40 (*m*)	33.9
CH_2(16)	0.90–0.96 (*m*), 1.58–1.68 (*m*)	28.1
H–C(17)	1.56–1.65 (*m*)	51.0
Me(18)	0.65 (*s*)	14.4
Me(19)	1.04 (*s*)	22.2
H–C(20)	1.38–1.42 (*m*)	36.4
Me(21)	0.90 (*d*, *J* = 6.6)	18.5
CH_2(22)	0.97–1.02 (*m*), 1.78–1.82 (*m*)	33.6
CH_2(23)	1.18–1.24 (*m*), 1.62–1.70 (*m*)	28.9
H–C(24)	3.43 (*d*, *J* = 11.2)	79.3
C(25)	–	73.9
CH_2(26)	3.47 (*d*, *J* = 11.4), 3.83 (*d*, *J* = 11.4)	67.7
Me(27)	1.10 (*s*)	20.9
Me(28)	0.80 (*s*)	16.4
Me(29)	0.97 (*s*)	28.2
Me(30)	0.74 (*s*)	18.5
MeO	3.37 (*s*)	57.5

[a]) Recorded at 400 (^{1}H-NMR) and 100 (^{13}C-NMR) MHz in $CDCl_3$, δ in ppm, *J* in Hz.

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Experimental Part

General. All solvents were of anal. grade (*Shanghai Chemical Plant*, Shanghai, P. R. China). Silica gel (SiO_2; 200–300 mesh) was used for column chromatography (CC), and precoated silica gel *GF254* plates (*Qingdao Haiyang Chemical Plant*, Qingdao, P. R. China) were used for TLC. *C18* reversed-phase (RP) silica gel (150–200 mesh, *Merck*), *MCI* gel (*CHP20P*, 75–150 μm, *Mitsubishi Chemical Industries Ltd.*), and *Sephadex LH-20* gel (*Amersham Biosciences*) were also used for CC. Optical rotations: *Perkin-Elmer 341* polarimeter. IR Spectra: *Perkin-Elmer 577* spectrometer with KBr disks. NMR Spectra: *Bruker AM-400* spectrometer, with TMS as internal standard. EI-MS (70 eV): *Finnigan MAT95* mass spectrometer.

Plant Material. The twigs and needles of *Pinus yunnanensis* were collected from Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Sciences, Mengla County, Yunnan Province, P. R. China, in October 2005, and were authenticated by Prof. *You-Kai Xu*, XTBG, Chinese Academy of Sciences. A voucher specimen has been deposited with Shanghai Institute of Materia Medica, SIBS, Chinese Academy of Sciences (accession No. PY-2005-1Y).

Extraction and Isolation. The air-dried and powdered twigs and needles of *Pinus yunnanensis* (4.7 kg) were percolated with 95% EtOH at r.t. After removal of the solvent under reduced pressure, the crude extract (300 g) was dispersed in H_2O, which was partitioned with AcOEt to give a dark viscous residue (135 g). This gum was subjected to an *MCI* gel *CHP20P* column eluted with MeOH/H_2O (0 to 90%) to afford three fractions, *1–3*. *Fr. 2* was separated on a SiO_2 column eluted with a mixture of petroleum ether (PE)/acetone 20:1 to 8:1 to give five fractions, *2a–2e*. *Fr. 2c* was subjected to a SiO_2 column eluted with a mixture of PE/AcOEt 15:1 to 10:1 to give three fractions, *2c1–2c3*. *Fr. 2c1* was purified by a SiO_2 column with a mixture of PE/acetone 15:1 to afford **1** (7 mg). *Fr. 2c2* was separated on a *C18* RP SiO_2 column eluted with 70% MeOH, and successively purified on *Sephadex LH-20* eluted with MeOH to yield **2** (2 mg) and junicedric acid (35 mg). 12-Hydroxydehydroabietic acid was recrystallized in acetone from *Fr. 2d* separated by the *C18* RP SiO_2 column (80% MeOH). *Fr. 3* was separated on a SiO_2 column eluted with a mixture of PE/acetone 20:1 to 6:1 to give six major fractions, *3a–3f*. *Fr. 3a* was purified by prep. HPLC with the mobile phase of 80% MeOH in H_2O (at flow rate of 3 ml/min) to give angustanoic acid D and 7,8-epoxy-1(12)-caryophyllen-9β-ol. *Fr. 3e* was subjected to *Sephadex LH-20* CC with MeOH to obtain a major mixture, which was then separated on a SiO_2 column eluted with CH_2Cl_2/MeOH 150:1 to afford **4** (10 mg). *Fr. 3f* was resubjected to a SiO_2 column eluted with CH_2Cl_2/MeOH 100:1 to 50:1 to afford **3** (7 mg).

15-Methoxydehydroabietic Acid (=*15-Methoxyabieta-8,11,13-trien-18-oic Acid*; **1**). Colorless gum. $[\alpha]_D^{20} = +51.0$ ($c = 0.10$, MeOH). UV (MeOH): 209 (3.96), 254 (2.56). IR (KBr): 3427, 2958, 1695, 1462, 1385, 1279, 1148, 706. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 330 (5), 315 (100), 299 (8), 283 (4), 269 (2), 237 (3), 149 (4), 73 (6). HR-ESI-MS: 353.2083 ($[M+Na]^+$, $C_{21}H_{30}NaO_3^+$; calc. 353.2195).

10-Hydroxy-9,10-secoabieta-8,11,13-trien-18-oic Acid (=rel-*(1*R*,2*S*,3*R*)-3-Hydroxy-1,3-dimethyl-2-{2-[3-(1-methylethyl)phenyl]ethyl}cyclohexanecarboxylic Acid*; **2**). Colorless gum. $[\alpha]_D^{20} = -17.0$ ($c = 0.10$, MeOH). UV (MeOH): 200 (4.58), 214 (3.96), 259 (2.90). IR (KBr): 3423, 2931, 1693, 1458, 1379, 1257, 1173, 1074, 827. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 300 (3), 146 (100), 131 (27), 117 (8), 109 (6), 91 (7), 81 (4), 55 (2). HR-ESI-MS: 341.2102 ($[M+Na]^+$, $C_{20}H_{30}NaO_3^+$; calc. 341.2195).

Pinusyunnanol (=rel-*(3*β*,24*S*)-3-Methoxylanost-9(11)-ene-24,25,26-triol*; **3**). Colorless solid. $[\alpha]_D^{20} = +59.0$ ($c = 0.09$, MeOH). IR (KBr): 3408, 2943, 2868, 1618, 1458, 1377, 1082. ^{1}H- and ^{13}C-NMR: *Table 2*. EI-MS: 490 (33, M^+), 475 (53), 443 (100), 425 (46), 399 (32), 367 (40), 159 (62), 95 (65). HR-EI-MS: 490.4033 (M^+, $C_{31}H_{54}O_4^+$; calc. 490.4022).

REFERENCES

[1] H. T. A. Cheung, T. Miyase, M. P. Lenguyen, M. A. Smal, *Tetrahedron* **1993**, *49*, 7903.
[2] A. H. Conner, J. W. Rowe, *Phytochemistry* **1977**, *16*, 1777.
[3] M. G. de Carvalho, V. M. Rumjanek, M. de J. S. Lopes, A. G. de Carvalho, *Phytochemistry* **1998**, *49*, 1101.
[4] M. D. Sutherland, J. W. Wells, *J. Org. Chem*. **1956**, *21*, 1272.
[5] R. A. Franich, J. K. Volkman, *Phytochemistry* **1982**, *21*, 2687.
[6] M. Z. Sultan, Y.-M. Jeon, S.-S. Moon, *Planta Med*. **2008**, *74*, 449.
[7] T. Minami, S. Wada, H. Tokuda, G. Tanabe, O. Muraoka, R. Tanaka, *J. Nat. Prod*. **2002**, *65*, 1921.
[8] Y. Jiang, B. T. Li, Y. H. Li, in 'Flora of China' ('Zhongguo Zhiwu Zhi'), Eds. Y. Jiang, B. T. Li, Science Press, Beijing, 1979, Vol. 7, p. 255.
[9] J. P. Kutney, G. Eigendorf, B. R. Worth, J. W. Rowe, A. H. Conner, B. A. Nagasampagi, *Helv. Chim. Acta* **1981**, *64*, 1183.
[10] L. K. Sy, G. D. Brown, *J. Nat. Prod*. **1998**, *61*, 907.

[11] H. Heymann, Y. Tezuka, T. Kikuchi, S. Supriyatna, *Chem. Pharm. Bull.* **1994**, *42*, 138.
[12] W.-C. Su, J.-M. Fang, Y.-S. Cheng, *Phytochemistry* **1996**, *41*, 255.
[13] Y. Kinouchi, H. Ohtsu, H. Tokuda, H. Nishino, S. Matsunaga, R. Tanaka, *J. Nat. Prod*. **2000**, *63*, 817.

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