Torreyanoxane, a New 3,4-Secoglutinane Triterpenoid Isolated from the Pulp of *Torreya nucifera*

by **Cong-Mei Cao**[a][b], **Yi-Bing Wu**[a], **Rui-Xia Guo**[a], **Mei Dong**[c], **Françoise Sauriol**[d], **Chang-Hong Huo**[a], **Qing-Wen Shi***[a][c], **Teiko Yamada**[e], **Hiromasa Kiyota***[e], **Yu-Cheng Gu**[f], and **Bin Cong***[c]

[a]) School of Pharmaceutical Sciences & College of Basic Medicine, Hebei Medical University, 361 Zhongshan East Road, Shijiazhuang 050017, P. R. China (phone: +86-311-86265634; e-mail: shiqingwen@hebmu.edu.cn)

[b]) Department of Horticultural Science, Texas A & M University, College Station, Texas, USA

[c]) Department of Forensic Medicine, Hebei Medical University, Hebei Key Laboratory of Forensic Medicine, Shijiazhuang 050017, P. R. China (phone: +86-311-6266402; e-mail: hbydcongbin@hebmu.edu.cn)

[d]) Department of Chemistry, Queen's University, Kingston, Ontario K7L3N6, Canada

[e]) Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiya, Aoba-ku, Sendai, 981-8555 Japan (phone/fax: +81-22-717-8785; e-mail: kiyota@biochem.tohoku.ac.jp)

[f]) Syngenta Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, UK

Torreyanoxane, a novel 3,4-secoglutinane triterpenoid, was isolated from the pulp of *Torreya nucifera*. The structure was determined on the basis of spectroscopic methods.

Introduction. – *Torreya nucifera* is an evergreen coniferous tree, which is common in Japan, Korea, and China. Its seeds exhibit significant insecticidal activity. The fruits of this evergreen, not only as a delicious food, are widely used in folk medicine for the treatment of tapeworm infestation in Korea, to prevent atherosclerosis in China, and to induce abortion in Japan, but most chemical studies of *T. nucifera* have been focused on its leaves and wood, which resulted in the isolation of a number of sesquiterpenoids, labdane and abietane diterpenoids, lignans, and flavonoids [1–8]. So, we investigated the chemical composition of the pulp of *T. nucifera*, and isolated a new 3,4-secoglutinane triterpenoid (*Fig. 1*).

Results and Discussion. – Compound **1** was isolated as an optically active amorphous solid. The molecular formula was elucidated as $C_{31}H_{52}O_2$ by analysis of the

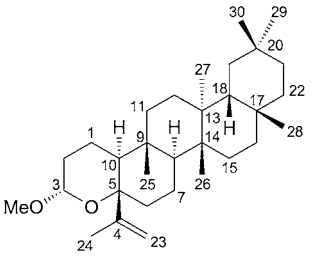


Fig. 1. *Structure of* **1**

HR-MS and ^{13}C-NMR data (*Table*), indicating the presence of six C=C bond/ring equivalents. The ^{1}H-NMR spectrum of **1** indicated the presence of six quaternary, one allylic, and one ethereal Me groups, in addition to one O–CH and two exocyclic methylidene H-atoms. The presence of eleven CH_2, three CH, and one O–CH C-atoms were elucidated by the DEPT analysis. The signals of the remaining six sp^3 and two sp^2 quaternary C-atoms were also detected in the ^{13}C-NMR spectrum. Accordingly, the whole structure seemed to be a triterpenoid containing five rings, one C=C bond, and one MeO group. Two sets of ^{1}H,^{1}H-COSY correlations from H–C(3) deshielded by two O-atoms (δ(H) 4.52 (*dd*, J = 9.4, 2.6) and from H–C(6) (δ(H) 2.44 – 2.48) revealed the connectivity C(3)–C(2)–C(1)–C(10) and C(6)–C(7)–C(8), respectively (*Fig. 2*). HMBCs from Me(24) to C(4), C(5), and C(23) indicated that this allylic Me- and exocyclic methylidene-containing an isopropenyl group was located at the O-bearing C(5)-atom (δ(C) 80.1). The presence of the angular Me(25) group at C(9) was elucidated by HMBCs of Me(25) to C(8), C(9), C(10), and C(11). HMBCs from H–C(6) to C(5) and C(23) indicated the presence of pyran (acetal) and cyclohexane rings fused along C(5)–C(10). Similarly, the entire C-atom connectivity was deduced by HMBC analysis from the angular (Me(26), Me(27), and Me(28)) and the geminal (Me(29) and Me(30)) Me groups. The relative configurations of the stereogenic centers at C(3), C(5), C(9), and C(11) were determined by means of H,H-coupling constant values and NOESY analyses (*Fig. 3*). Since NOESY correlations could not be further analyzed due to overlapping of the CH_2 signals, the relative configurations at the other positions were proposed by comparing the NMR data with that of terminalin A, the first 3,4-secoglutinane isolated from the stem bark of *Terminalia glaucescens* [9]. The structure of **1** was thus deduced as 3β,5α-epoxy-3α-methoxy-3,4-secoglutin-4-ene, designated as torreyanoxane.

Table. *^{1}H- and ^{13}C-NMR* (500 and 125 MHz, resp.) *Data of* **1** *in (D_5)Pyridine.* δ in ppm, J in Hz.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
1	1.58 – 1.63, 1.75 – 1.79 (2*m*)	18.8	17	–	30.1
2	1.62 – 1.66, 1.94 – 1.97 (2*m*)	33.4	18	1.50 – 1.54 (*m*)	42.9
3	4.52 (*dd*, J = 9.4, 2.6)	98.6	19[a])	1.18 – 1.23 (*m*)	35.7
4	–	143.6	20	–	28.3
5	–	80.1	21[c])	1.13 – 1.17, 1.34 – 1.38 (2*m*)	33.0
6	1.56 – 1.61 (*m*), 2.44 – 2.48 (*s*)	40.6	22[d])	0.88 – 0.92, 1.43 – 1.48 (2*m*)	39.5
7	1.09 – 1.13 (*m*), 1.40 (*s*)	18.7	23	5.35, 5.29 (2*s*)	119.6
8	1.52 (*s*)	54.8	24	1.89 (*s*)	21.6
9	–	38.2	25	0.93 (*s*)	21.1
10	1.78 – 1.83 (*m*)	60.4	26	0.92 (*s*)	20.9
11[a])	1.32 – 1.36, 1.37 – 1.42 (2*m*)	35.7	27	0.97 (*s*)	18.8
12	1.16 – 1.21, 1.25 – 1.29 (2*m*)	31.1	28	1.15 (*s*)	32.5
13[b])	–	38.4	29	0.98 (*s*)	35.2
14[b])	–	40.2	30	1.05 (*s*)	31.9
15[c])	1.25 – 1.29, 1.43 – 1.47 (2*m*)	33.1	MeO	3.44 (*s*)	55.7
16[d])	1.28 – 1.32, 1.48 – 1.53 (2*m*)	36.3			

[a]) – [d]) Exchangeable signals.

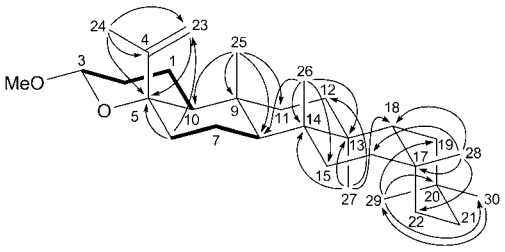


Fig. 2. *Key HMB* (H → C) *and* $^1H,^1H$*-COSY correlations* (—) *for* **1**

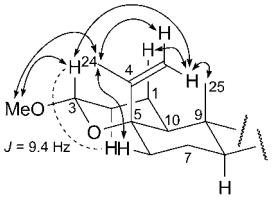
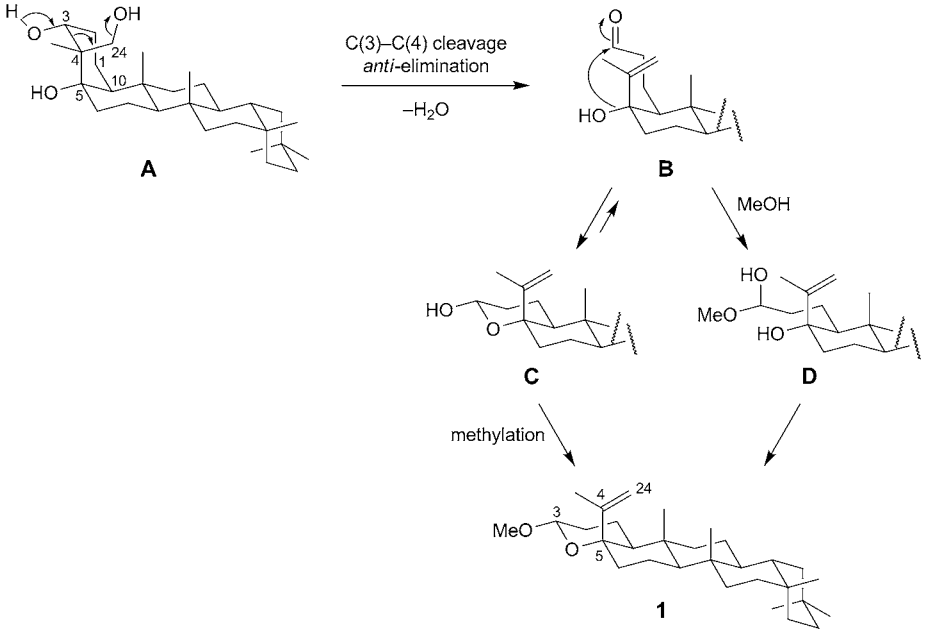


Fig. 3. *Key NOESY correlations* (H ↔ H) *for* **1**

A biogenetic pathway from a glutinane-3α,5α,24-triol (**A**) to **1** is proposed (*Scheme*). Concerted dehydrative *anti*-elimination cleaves the C(3)–C(4) bond of **A** to form hydroxy aldehyde **B**. Hemiacetal formation, followed by S_N2-type methylation (*via* **C**) or methyl acetal formation, and acetal exchange (*via* **D**) affords **1**. The MeO group is located in anomerically unstable equatorial position avoiding the interaction with the bulky isopropenyl group.

Scheme. *Plausible Biosynthetic Pathway to* **1**



Experimental Part

General. Flash chromatography (FC): silica gel *60* (SiO_2; 230–400 mesh; *EM Science*). TLC: SiO_2 *60* F_{254} (0.25 or 0.5 mm; *EM Science*). Prep. HPLC: *Waters Delta Prep 3000*, *Waters UV 486* tunable absorbance detector (210 nm), and *Whatman partisil 10 ODS-2 Mag-9* (9.4 × 250 mm). Optical rotation: *Jasco DIP-370*. NMR: *Bruker Avance-500* in (D_5)pyridine$_5$; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS: *Vacuum Generators ZAB-HS*; in *m*/*z*.

Plant Material. The pulp of *Torreya nucifera* was collected in the autumn of 1998 on Aoba mountain of Sendai city in Northeast of Japan. The botanical identification was accomplished by Prof. *Takashi Oritani* at the Toyama Prefectural University, Toyama, Japan. A voucher specimen with access No. 1998-11-1 had been deposited with the Laboratory of Applied Bioorganic Chemistry, Graduate School of Agricultural Sciences, Tohoku University, Japan.

Extraction and Isolation. Air-dried pulp (1.3 kg) of *Torreya nucifera* was extracted with 10 l of MeOH at r.t. The combined org. extracts were evaporated under reduced pressure. H_2O (1 l) was added, and lipids were removed by stirring the mixture with hexane (3 × 1 l). The aq. phase was then salted and extracted with CH_2Cl_2. The combined CH_2Cl_2 extract was dried (Na_2SO_4), filtered, and evaporated to yield a translucent yellow extract. A portion of the CH_2Cl_2 extract (31.2 g) was subjected to column chromatography (CC). Successive stepwise elution with petroleum ether/AcOEt gradient (10 : 7 to 10 : 2) yielded 40 fractions, *Frs. 1–40*. *Fr. 13* was applied to prep. HPLC, eluted with a 50 min linear gradient of MeCN (25 to 100%) in H_2O (3 ml/min), to give compound **1** (t_R 33.3 min).

Torreyanoxane (= *3β,5α-Epoxy-3α-methoxy-3,4-secoglutin-4-ene* = (*3*S,*4a*S,*6a*S,*6b*R,*8a*R,*12a*R,*12b*-S,*14a*R,*14b*S)-*Icosahydro-3-methoxy-6b,8a,11,11,12b,14a-hexamethyl-4a-(1-methylethenyl)-1*H-*chryseno[2,1-*b*]pyran*; **1**). Yield *ca.* 3.5 mg. White amorphous solid. $[\alpha]_D^{22} = +43$ ($c = 0.050$, MeOH). NMR: *Table*. HR-FAB-MS: 495.3608 ($[M+K]^+$, $C_{31}H_{52}KO_2^+$; calc. 495.3604).

The authors gratefully acknowledge the financial supports from the *National Natural Science Foundation of China* (81072551), the *Key Projects of Science and Technology of Hebei Province* (11276103D-89), the *Scientific Research Foundation for the Returned Overseas Chinese Scholars of Hebei Province* (2006-02), and the *Scientific Research Foundation of Hebei Province* (08B032 and C2010000489). We also wish to extend our sincere thanks for the financial support from *Syngenta Ltd.* (2011-Hebei Medical University-Syngenta-03) and the *Japan Society for the Promotion of Science* (Nos. 19580120 and 22580112).

REFERENCES

[1] I. Fukushima, Y. Sayama, K. Kyogoku, H. Murayama, *Agric. Biol. Chem.* **1968**, *32*, 1103.
[2] L. J. Harrison, Y. Asakawa, *Phytochemistry* **1987**, *26*, 1211.
[3] T. Sakai, K. Nishimura, Y. Hirose, *Bull. Chem. Soc. Jpn.* **1965**, *38*, 381.
[4] Y. Sayama, K. Kyogoku, H. Murayama, *Agric. Biol. Chem*. **1971**, *35*, 1068.
[5] T. Kariyone, T. Sawaka, *Yakugaku Zashi* **1958**, *78*, 1010.
[6] Y. P. Jang, S. R. Kim, Y. C. Kim, *Planta Med.* **2001**, *67*, 470.
[7] T. Sakai, K. Nishimura, Y. Hirose, *Tetrahedron Lett*. **1963**, *18*, 1171.
[8] Y. Orihara, J. W. Yang, N. Komiya, K. Koge, T. Yoshikawa, *Phytochemistry* **2002**, *59*, 385.
[9] Atta-ur-Rahman, S. Zareen, M. I. Choudhary, F. N. Ngounou, A. Yasin, M. Parvez, *Tetrahedron Lett.* **2002**, *43*, 6233.

Received April 5, 2012