Terpenoids of *Syzygium formosanum*

Churng-Werng Chang,[†] Tien-Shung Wu,[‡] Yih-Shou Hsieh,[§] Sheng-Chu Kuo,[†] and Pei-Dawn Lee Chao*[,†]

Institute of Pharmaceutical Chemistry, China Medical College, Taichung, Taiwan, Republic of China

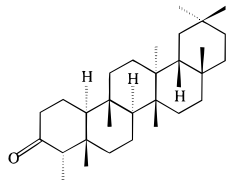
Received July 17, 1998

A new natural product, 4-epifriedelin (*1*), and 12 known terpenoids have been isolated from the leaves of *Syzygium formosanum*. The known compounds include caryophyllene oxide, friedelin, canophyllal, glutinol, α-terpineol, phytol, betulinic acid, uvaol, lupeol, betulin, ursolic acid, and oleanolic acid. All of these compounds are reported for the first time from S. *formosanum*.

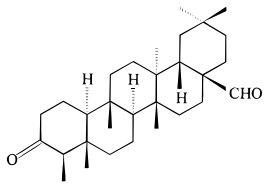
Syzygium formosanum (Hay.) Mori (Myrtaceae) is endemic to Taiwan. In the course of our continuing search for anti-HIV agents from natural products, a 70% acetone extract of the leaves demonstrated promising inhibition of Moloney murine leukemia virus (Mo-MuLV) reverse transcriptase. The acetone extract was partitioned between H_2O and CH_2Cl_2, and the CH_2Cl_2 fraction still showed inhibitory effects on Mo-MuLV reverse transcriptase. This study reports the isolation of 13 terpenoids from this fraction, all of them reported for the first time from this plant, of which **1** is a new natural product.

Compound **1** was obtained as colorless needles. Its IR spectrum exhibited a band for carbonyl (1717 cm $^{-1}$). The ^{13}C NMR spectrum of compound **1** revealed chemical shifts of carbons on C, D, and E rings very similar to those of friedelin, but two methyl carbons were found further downfield than friedelin (δ 13.5, 23.1 vs δ 6.8, 14.6). HMQC of **1** revealed that the proton attached to the carbon at δ 13.5 was a doublet (J = 7.3 Hz), indicating this carbon was assignable to C-23. Based on the above data, compound **1** appeared to be the C-4 epimer of friedelin. Upon standing in $CDCl_3$ for one month after NMR measurement, compound **1** had its ^{13}C NMR spectrum recorded again, it indicated the coexistence of friedelin[1] with compound **1** (ratio ca. 1:1). The mixture was then separated again by HPLC to afford compound **1** and friedelin. Therefore, the structure of **1** was determined to be 4-epifriedelin, a compound not reported previously as a natural product. The structure of **1** was further confirmed by ^{1}H−^{1}H COSY, HMBC, and NOESY techniques. The epimerization of **1** at C-4 in solvent could be accounted for by keto−enol tautomerism of the ketone group at C-3, and the equilibrium favored the more stable configuration of friedelin. 4-Epifriedelin had been previously reported as a product obtained by photoepimerization of friedelin.[2]

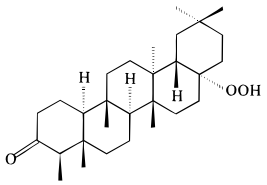
Canophyllal (**2**) was obtained as a yellow powder. It showed 30 carbons, including two carbonyls (δ 213.4 and 209.1) in the ^{13}C NMR spectrum and an aldehyde group in ^{1}H NMR spectum (δ 9.48, s, 1H). Upon standing in $CDCl_3$ for four months, compound **2** was subjected to ^{13}C NMR again, and it was found to have transformed into a different compound that showed only 29 carbons, including one carbonyl at δ 213.4. HMQC and HMBC analyses revealed that **3** was of the friedelane type. By comparison with the ^{13}C NMR spectrum of friedelin, **3** had one less methyl, and one carbon at δ 82.7 was shifted downfield from δ 30.0 in comparison to friedelin. The IR spectrum of **3** showed hydroxyl absorption at 3481 cm^{-1} . EIMS exhibited a significant peak at 426 ($M^+ - H_2O$). Based on the above data, **3** was determined as maytensifolin A. Comparison of the ^{1}H NMR, ^{13}C NMR, and EIMS with those of maytensifolin A in the literature confirmed this asssignment.[3−5] As to the structure of **2,** its ^{1}H NMR and MS spectral data were identical with those reported for canophyllal.[3] The spontaneous transformation of canophyllal to maytensifolin A has not been reported previously, and the mechanism remains to be elucidated.



1



2



3

Based on the ^{1}H NMR, ^{13}C NMR, and MS data, together with their physical constants, the other isolates were characterized as caryophyllene oxide,[6] friedelin,[1] glutinol,[1] α-terpineol,[7] phytol,[8] betulinic acid,[5,9,10] uvaol,[5,11] lupeol,[5,12] betulin,[5,9,10] ursolic acid,[13] and oleanolic acid. [5,13,14]

Bioactivity tests of the isolates against Mo-MuLV reverse transcriptase indicated that betulinic acid which has previously been reported to show an EC_{50} of 1.4 μM,[15] was a promising inhibitor of human immunodeficiency virus (HIV).

[†] China Medical College.
[‡] Cheng Kung University.
[§] Chung-Shan Medical and Dental College.
* To whom correspondence should be addressed. Fax: 886 4 2032146. E-mail: pdlee@cmce.cmc.edu.tw.

10.1021/np980313w CCC: $18.00
Published on Web 01/05/1999

Experimental Section

General Experimental Procedures. The preparative HPLC apparatus was equipped with a Shimadzu LC-10S pump and a Waters 410 differential refractometer. LiChrospher Si 60 column (10 μm, 25 cm × 1 cm) was used for separations. Melting points were measured on a Yanaco MP-500 and are uncorrected. UV spectra were obtained on a Shimadzu UV-160A, whereas IR spectra were recorded on a Nicolet Impact 400 FT-IR. EIMS were obtained on a Hewlett−Packard 5995 GC-MS system by direct probe (70 ev). NMR spectra were recorded on Bruker ARX-200, ARX-300 or AMX-400 FT-NMR spectrometers. Optical activities were measured on a JASCO DIP-1000 polarimeter.

Plant Material. The leaves of *Syzygium formosanum* (4 kg) were collected in Taipei in September 1994, and identified by Dr. Chung-Chuan Chen of the Institute of Chinese Pharmaceutical Sciences, China Medical College.

Extraction and Isolation. The air-dried leaves were blended with 70% Me_2CO in a juicer. After filtration, Me_2CO was removed by concentration under reduced pressure. The residue was suspended in H_2O and partitioned six times with CH_2Cl_2 to afford an aqueous fraction (700 g) and a CH_2Cl_2 fraction (105 g).

The CH_2Cl_2 fraction was chromatographed on a Si gel column and eluted with increasing amounts of EtOAc in *n*-hexane. Further separation was carried out by using Si gel HPLC, which employed isocratic elution with various percentages of EtOAc in *n*-hexane as mobile phases. The chromatographed fraction yielded caryophyllene oxide (15 mg), friedelin (600 mg), 4-epifriedelin (**1**, 165 mg), canophyllal (**2**, 10 mg), glutinol (35 mg), α-terpineol (15 mg), phytol (20 mg), betulinic acid (35 mg), uvaol (25 mg), lupeol (30 mg), betulin (90 mg), ursolic acid (20 mg), and oleanolic acid (15 mg).

Reverse Transcriptase Assay. The aforementioned isolates were subjected to an in vitro reverse transcriptase assay. Both $(\gamma A)_n$-$(dT)_{15}$ and unlabeled TTP were obtained from Boehringer Mannheim GmbH, Penzber, Germany. ^{3}H-TTP was purchased from Amersham Co. Reverse transcriptase from Moloney murine leukemia virus (Mo-MuLV) is the product of Bethesda Research Laboratories Life Technologies, Inc., Gaithersburg, MD. The assay reaction mixture (30 μL) contained 50 mM Tris−HCl buffer, pH 8.3; 75 mM KCl; 10 mM dithiothreitol; 3 mM $MgCl_2$; 0.5 μg of $(\gamma\ A)_n$-$(dT)_{15}$; 0.5 μ Ci [^{3}H]-dTTP, and 5 units of Mo-MuLV reverse transcriptase preparation.[16] After incubation at 37 °C for 30 min, 5 μL of 0.4 M EDTA was added to terminate the reaction. The reaction mixture (15 μL, in triplicate) was spotted on Whatman DEAE cellulose paper and washed three times each with 5% Na_2HPO_4, H_2O, and EtOH. The radioactivity of the sample was measured using a Packard 2200 CA, Tri-carb Liquid Scintillation Counter.

4-Epifriedelin (1): colorless needles (*n*-hexane−EtOAc); mp 242−245 °C, $[\alpha]_D$ +2.7° (*c* 0.19, $CHCl_3$,), IR ν_{max} (KBr) cm^{-1} 2944, 2863, 1717, 1460, 1381, 1206, 1193, 1058, 978, 953; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.80 (3H, s, H-25), 0.85 (1H, m, H-22a), 0.86 (3H, s, H-24), 0.88 (3H, s, H-29), 0.93 (3H, s, H-30), 0.94 (3H, s, H-26), 0.98 (3H, s, H-27), 1.05 (3H, d, J = 7.3 Hz, H-23), 1.11 (3H, s, H-28), 1.14 (1H, m, H-6a), 1.16 (2H, m, H-19), 1.23 (1H, m, H-15a), 1.27 (1H, m, H-8), 1.29 (1H, m, H-12), 1.31 (1H, m, H-11a), 1.36 (1H, m, H-11b), 1.44 (1H, m, H-22b), 1.45 (1H, m, H-15b), 1.47 (1H, m, H-18), 1.54 (2H, m, H-1a and H-10),1.55 (1H, m, H-6b), 1.77 (1H, m, H-1b), 1.84 (1H, m, H-4), 2.24 (1H, m, H-2a), 2.44 (1H, m, H-2b); ^{13}C NMR ($CDCl_3$, 100 MHz) C-1 21.7, C-2 37.1, C-3 216.6, C-4 58.7, C-5 39.9, C-6 37.4, C-7 17.7, C-8 53.5, C-9 37.0, C-10 49.4, C-11 35.7, C-12 30.5, C-13 39.7, C-14 38.3, C-15 32.4, C-16 36.0, C-17 30.0, C-18 42.7, C-19 35.3, C-20 28.1, C-21 32.7, C-22 39.2, C-23 13.5, C-24 23.1, C-25 18.0, C-26 20.4, C-27 18.7, C-28 32.1, C-29 35.0, C-30 31.7; EIMS (*m*/*z*, %) 426 [M$^+$] (8), 341 (3), 302 (7), 273 (12), 246 (12), 205 (17), 179 (19), 163 (31), 137 (26), 125 (44), 123 (53), 109 (68), 95 (100), 81 (71), 69 (91), 55 (72).

References and Notes

(1) Akihisa, T.; Yamamnoto, K.; Tamura, T.; Kimura, Y.; Iida, T.; Nambara, T.; Chang, C. F. *Chem. Pharm. Bull.* **1992**, *40*, 789−791.
(2) Aoyaki, R.; Yamda, S.; Tsuyuki, T.; Takahashi, T. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 959−963.
(3) Nozaki, H.; Suzuki, H.; Hirayama, T.; Kasai, R.; Wu, R. Y.; Lee, K. H. *Phytochemistry* **1986**, *25*, 479−485.
(4) Patra, A.; Chaudhuri, S. K. *Magn. Reson. Chem.* **1987**, *25*, 95−100.
(5) Mahato, S. B.; Kundu, A. P. *Phytochemistry* **1994**, *37*, 1517−1575.
(6) Sashida, Y.; Itokawa, H.; Akita, Y.; Fujita, M. *Yakugaku Zasshi* **1976**, *96*, 218−222.
(7) Harada, R.; lwasaki, M. *Phytochemistry* **1982**, *21*, 2009−2011.
(8) Suga, T.; Aoki, T. *Phytochemistry* **1974**, *13*, 1623−1624.
(9) Salimuzzaman, S.; Farrukh, H.; Sabira, B.; Bina, S. S. *J. Nat. Prod.* **1988**, *51*, 229−233.
(10) Sholichin, M.; Yamasaki, K.; Kasai, R.; Tanaka, O. *Chem. Pharm. Bull.* **1980**, *28*, 1006−1008.
(11) Salimuzzaman, S.; Farrukh, H.; Sabira, B.; Bina, S. S. *J. Nat. Prod.* **1986**, *49*, 1086−1090.
(12) William, F. R.; Stewart, M.; Janusz, P. *Tetrahedron* **1986**, *42*, 3419−3428.
(13) Tsutomu, F.; Yutaka, O.; Chisato, H. *Phytochemistry* **1987**, *26*, 715−719.
(14) Maillard, M.; Adewunmi, C. O.; Hostettmann, K. A. *Phytochemistry* **1992**, *31*, 1321−1323.
(15) Fujioka, T.; Kashiwada, Y.; Kilkuskie, R. E.; Cosentino, L. M.; Ballas, L. M.; Jiang, J. B.; Janzen, W. P.; Chen, I. S.; Lee, K. H. *J. Nat. Prod.* **1994**, *57*, 243−247.
(16) Chu, S. C.; Hsieh, Y. S.; Lin, J. Y. *J. Nat. Prod.* **1992**, *55*, 179−183.

NP980313W