Anwuweizonic acid and manwuweizic acid, the putative anticancer active principle of *Schisandra propinqua*

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This paper is dedicated to the memory of Professor Karel Wiesner

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The structures of two new triterpenoid acids, anwuweizonic acid (**1**) and manwuweizic acid (**2**), from *Schisandra propinqua* are elucidated by spectroscopic evidence and chemical correlation. Compound **2** shows significant inhibitory activity against Lewis lung cancer, brain tumor-22, and solid hepatoma in mice, and exhibits no cytotoxic action in vitro.

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Faisant appel à des données spectroscopiques et à des corrélations chimiques, on a élucidé les structures de deux nouveaux acides, les acides anwuweizonique (**1**) et manwuweizique (**2**), qui ont été isolés du *Schisandra propinqua*. Le composé **2** présente une activité inhibitrice importante vis-à-vis du cancer des poumons de Lewis, du tumeur 22 du cerveau et de l'hépatome solide chez la souris et il ne présente pas d'activité cytotoxique in vitro.

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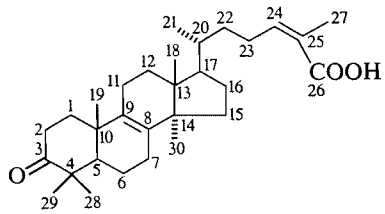
It has been reported that Manshanxiang Complex, a herbal medicine preparation in which the stems and roots of *Schisandra propinqua* (Wall.) Hook. f. et Thoms are a major component, was used for treatment of lung carcinoma in several hospitals of Yunnan Province in China (1), and that the water extract from the stems and roots of *S. propinqua* shows activity against Lewis lung cancer in animal tests (2). To isolate the active principle, we started chemical studies on this plant. Two new triterpenoid acids, anwuweizonic acid (**1**) and manwuweizic acid (**2**), were isolated from its alcoholic extract and the second (**2**) exhibits significant inhibitory activity against Lewis lung cancer, brain tumor-22, and solid hepatoma in mice and shows no cytotoxic action in vitro.[2] Therefore, **2** is probably a major anticancer active principle of the plant under study. In this paper we wish to report the structural elucidation of the two acids.

Anwuweizonic acid (**1**), $C_{30}H_{46}O_3$, gave a monoester on reaction with diazomethane. The ^1H nmr spectrum of **1** reveals the presence of a moiety of the angelic acid terminal (3) (δ 6.10, 1H, t, J = 7, H-24; δ 1.94, 3H, s, H-27), a secondary methyl (δ 0.92, 3H, d, J = 5, H-21), and five tertiary methyls (δ 0.72, 0.90, 1.10, 1.14, 3H, 3H, 6H, 3H each, s), and its ir spectrum shows the characteristics of an α,β-unsaturated acid (1690, 3200–2500 cm^{-1}) and a ketone (1710 cm^{-1}). These features closely resemble those of anwuweizic acid (**3**) (4) except for the ketone group of **1** in place of a hydroxyl in **3**, suggesting that anwuweizonic acid possesses structure **1**. This proposal was verified by reduction of **1** with sodium borohydride in methanol, providing anwuweizic acid (**3**, 8% yield) and its 3β-hydroxyl isomer (85% yield).

Manwuweizic acid (**2**), $C_{30}H_{46}O_4$, furnished a diester on methylation with diazomethane, a feature of a dicarboxylic acid. The ^1H nmr spectrum of **2** shows the presence of an isopropenyl group (δ 4.91, 4.69, 1H each, s, H-28; δ 1.77, 3H, s, H-29) and three tertiary methyls (δ 0.74, 0.94, 0.97, 3H each, s, H-18, H-19, H-30) besides a moiety of the angelic acid terminal (δ 6.09, 1H, dd, J = 6.7/7.1, H-24; δ 1.91, 3H, s, H-27) and a secondary methyl (δ 0.96, 3H, d, J = 4.8, H-21) in the molecule. These facts indicate that manwuweizic acid possesses the 3,4-seco-ring A and a side chain identical to **1**, which led to the proposed structure **2** for manwuweizic acid.



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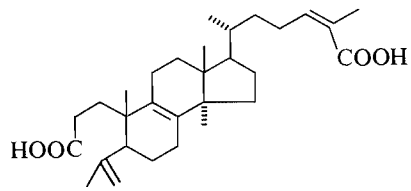

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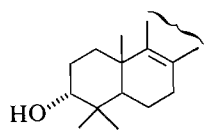

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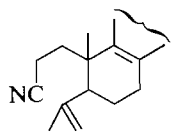

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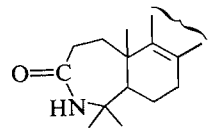

5

The following chemical correlation among anwuweizonic acid (**1**), manwuweizic acid (**2**), and anwuweizic acid (**3**), a known compound (4), further confirmed the proposed structures. Oxidation of **3** in ether with an aqueous solution of sodium dichromate – sulfuric acid provided anwuweizonic acid (**1**, 93% yield), and then oximation of **1** with hydroxylamine hydrochloride in ethanol–pyridine (1:1) afforded an oxime (98% yield). The oxime underwent an abnormal Beckmann rearrangement (5) in anhydrous pyridine and *p*-tosyl chloride at room temperature, giving a nitrile compound (**4**, 25% yield) and a lactam (**5**, 56% yield). Hydrolysis of **4** with 10% ethanolic KOH under reflux for 4 h led to **2** (70% yield), which is identical with natural manwuweizic acid. Therefore, the attribution of structure **1**, 3-oxo-lanosta-8,24*Z*-dien-26-oic acid, to anwu-

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weizonic acid and structure **2**, 3,4-secolanosta-4(28),8,24*Z*-trien-3,26-dioic acid, to manwuweizic acid was fully supported.

Experimental

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. The following instruments were used for the spectra described here: ir, Perkin–Elmer 599B with KBr as the medium; ^{1}H nmr, JEOL PS-100 (100 MHz), Bruker AM-400 (400 MHz); and ^{13}C nmr, Bruker AC-100 (25.18 MHz), with $CDCl_3$ as the solvent, δ in ppm, and *J* in Hz; ms, Varian MAT-711.

Manwuweizic acid (2)

The crude alcoholic extract of the roots and stems of *Schisandra propinqua* (Wall.) Hook. f. et Thoms, gathered in Yuxi District, Yunnan Province, China, was suspended in water and extracted with ether. Then the ether extract was chromatographed on a silica gel column eluting with petroleum ether/benzene (1:1), benzene, benzene/ethyl acetate (10:1 and 4:1). The fractions eluted with benzene/ethyl acetate (4:1) gave crude crystals of manwuweizic acid (**2**). Its mother liquor was rechromatographed over a silica gel column, again giving **2** (see the isolation of **1**), which was recrystallized from chloroform/petroleum ether as needles (yield 0.04%), mp 191–193°C; $[\alpha]_D^{15}$ +54.3° (*c* 0.291, $CHCl_3$); ir ν_{max}: 3200–2500, 1705, 1695, 1630 cm^{-1}; ^{1}H nmr (400 MHz) δ: 6.09 (1H, dd, *J* = 6.7/7.1, H-24), 4.91, 4.69 (1H each, s, H-28), 1.91 (3H, s, H-27), 1.77 (3H, s, H-29), 0.74, 0.94, 0.97 (3H each, s, H-18, H-30, H-19), 0.96 (3H, d, *J* = 4.8, H-21); ^{13}C nmr (25.18 MHz) δ: 179.91 (s, C-3), 172.94 (s, C-26), 147.47 (s, C-4), 146.81 (d, C-24), 139.59, 129.77 (s, C-9, C-8), 126.10 (s, C-25), 113.85 (t, C-28), 51.09, 44.75 (s, C-14, C-13), 50.48 (d, C-17), 47.45 (d, C-5), 40.66 (s, C-10), 36.46 (d, C-20), 36.02, 32.87, 31.46, 31.21, 29.74, 28.12, 26.86, 26.11, 24.37, 22.03 (t each, C-1, C-2, C-6, C-7, C-11, C-12, C-15, C-16, C-22, C-23), 18.74 (q, C-27), 20.40 (q, C-21), 22.29, 23.08, 16.16, 25.30 (q, C-18, C-19, C-29, C-30); ms *m/z*: 470.3402 (M^+, calcd. for $C_{30}H_{46}O_4$: 470.3396), 455.3195 (M − CH_3), 397.3078 (M − CH_2CH_2COOH), 261.1853 ($C_{17}H_{25}O_2$), 235.1703 ($C_{15}H_{23}O_2$), 233.1560 ($C_{15}H_{21}O_2$).

Anwuweizonic acid (1)

The fractions eluted with benzene/ethyl acetate (10:1) mentioned above and the mother liquor of **2** were combined and rechromatographed on a silica gel column using petroleum ether/acetone (10:1). The late fractions again gave **2** and the early fractions afforded anwuweizonic acid (**1**) (yield 0.012%), crystallized from ether/petroleum ether, mp 126–128°C; $[\alpha]_D^{15}$ +63.8° (*c* 0.273, $CHCl_3$); ir ν_{max}: 3200–2500, 1710, 1690, 1630 cm^{-1}; ^{1}H nmr (100 MHz) δ: 6.10 (1H, t, *J* = 7, H-24), 1.94 (3H, s, H-27), 0.92 (3H, d, *J* = 5, H-21), 0.72, 0.90, 1.10, 1.14 (3H, 3H, 6H, 3H each, s, H-18, H-30, H-19, H-28, H-29); ms *m/z*: 454.3445 (M^+, calcd. for $C_{30}H_{46}O_3$: 454.3447), 439.3192 (M − CH_3), 271.2082 ($C_{19}H_{27}O$).

Methyl ester of 1

Compound **1** (30 mg) in ether reacted with an ethereal solution of diazomethane affording the methyl ester (30 mg) of anwuweizonic acid, crystallized from methanol, mp 89–90°C; ir ν_{max}: 1720, 1705, 1650 cm^{-1}; ^{1}H nmr (100 MHz) δ: 3.73 (3H, s, $COOCH_3$); ms *m/z*: 468 (M^+), 453 (M − CH_3), 421.

Dimethyl ester of 2

The same procedure as described above provided the dimethyl ester of manwuweizic acid, amorphous, ir ν_{max}: 1740, 1720, 1650 cm^{-1}; ^{1}H nmr (100 MHz) δ: 3.60, 3.70 (3H each, s, $COOCH_3$ × 2); ms *m/z*: 498 (M^+), 483 (M − CH_3), 411 (M − $CH_2CH_2COOCH_3$).

Reduction of 1 to 3 and the 3β-hydroxyl isomer of 3

A solution of **1** (100 mg) in methanol (3 mL) with $NaBH_4$ (200 mg) was allowed to stand for 2 h at room temperature. The reaction mixture, after evaporation of the methanol in vacuum, was treated with water (5 mL) and one drop of hydrochloric acid, then extracted with chloroform. The extract was applied to a silica gel column eluting with benzene/ethyl acetate (10:1). The early fractions gave anwuweizic acid (**3**, 8 mg) identical with an authentic sample (4) (mixture mp, ir), and the late fractions afforded the 3β-hydroxyl isomer (85 mg) of **3**, mp 148–149°C, which is distinguished from **3** in the fingerprint region of ir and the H-3α (δ 3.24, 1H, dd, *J* = 4/9) signal of ^{1}H nmr.

Oxidation of 3 to 1

Compound **3** (45 mg) in ether (5 mL) was oxidized with an aqueous solution (0.6 mL) of sodium dichromate – sulfuric acid (1 g sodium dichromate in 6 mL water mixed with 1 mL 97% sulfuric acid, then diluted to 20 mL with water) under stirring for 5 h at room temperature. The ethereal layer was separated and the water layer was extracted with ether. The combined ethereal solution was washed with a saturated solution of sodium chloride and then concentrated, giving **1** (42 mg) identical to the natural compound (mixture mp and ir).

Oxime of 1

A mixture of **1** (300 mg) in pyridine (3 mL) and hydroxylamine hydrochloride (51 mg) in ethanol (3 mL) was heated on a boiling water bath for 1 h, and then diluted with water (100 mL). The solid product was filtered and crystallized from methanol, giving the oxime (300 mg), mp 184–186°C, ir ν_{max}: 3260, 3200–2500, 1690, 1640 cm^{-1}; ms *m/z*: 469 (M^+), 454 (M − CH_3), 436, 418.

Products (4 and 5) of Beckmann rearrangement

To a solution of the oxime (300 mg) in anhydrous pyridine (5 mL) was added *p*-tosyl chloride (300 mg), and the solution was allowed to stand for 24 h at room temperature. After dilution with water (50 mL) and acidification to pH 2 with hydrochloric acid, the reaction mixture was extracted using $CHCl_3$. The extract was chromatographed on a silica gel column eluting with benzene/ethyl acetate (10:1). The early fractions gave the nitrile compound (**4**, 70 mg), amorphous, ir ν_{max}: 3200–2500, 2250, 1690, 1640 cm^{-1}; ^{1}H nmr (100 MHz) δ: 6.10 (1H, t, *J* = 7, H-24), 4.95, 4.69 (1H each, s, H-28), 1.93 (3H, s, H-27), 1.77 (3H, s, H-29), 0.99, 0.95, 0.75 (3H each, s, H-19, H-30, H-18), 0.94 (3H, d, *J* = 4, H-21); ms *m/z*: 451 (M^+), 436 (M − CH_3), 418, 397.

The late fractions afforded the lactam (**5**, 170 mg), amorphous, ir ν_{max}: 3300, 3210, 3200–2500, 1690, 1640 cm^{-1}; ^{1}H nmr (100 MHz) δ: 5.97 (1H, t, *J* = 7, H-24), 1.92 (3H, s, H-27), 1.37, 1.31, 1.22, 0.92, 0.74 (3H each, s, H-29, H-28, H-19, H-30, H-18), 0.93 (3H, covered, H-21); ms *m/z*: 470 (*M* + 1), 469 (M^+), 455, 454, 398, 397, 379, 351.

Manwuweizic acid (2) from hydrolysis of 4

The nitrile compound (**4**) was dissolved in an ethanolic solution of 10% KOH and refluxed for 4 h. After evaporation of ethanol, the reaction mixture was acidified to pH 3 with hydrochloric acid and extracted with $CHCl_3$. The extract was purified on a silica gel column eluting with $CHCl_3$, giving **2** (50 mg) identical to the natural compound (mixture mp, ms, ^{1}H nmr, ir).

Acknowledgements

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