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Synthesis of Manwuweizic Acid, An Anticancer Triterpenoid

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This paper is dedicated to Professor William A. Ayer on the occasion of his 60th birthday

Abstract: Manwuweizic acid (**1**), an anticancer triterpenoid isolated from *Scisandra propinqua*, was synthesized from lanosterol (**2**) by stereoselective introduction of the (Z) α,β-unsaturated carboxylic terminal of the side chain and by abnormal Beckmann rearrangement of ring A. A new reagent of DMSO-DCC-TFA for protection of a carboxyl group as a methylthiomethyl (MTM) ester is also described.

In our previous paper[1] we reported the structure of manwuweizic acid (**1**), an triterpenoid, isolated from the anticancer Chinese ethno-medicine, *Schisandra propinqua* (Wall.) Hook. f. et Thoms. Manwuweizic acid (**1**) exhibits significant inhibitory activity against Lewis lung cancer, brain tumor-22 and solid hepatoma in mice, and shows no cytotoxic activity *in vitro*.[1] In order to evaluate its potential as a therepeutic agent, a large quantity of the compound **1** was required for animal tests. Since the content of this compound in the plant is rather low (<0.04%) and its separation from a minor toxic component is tedious, a synthetic approach to manwuweizic acid (**1**) was designed.

Manwuweizic acid (**1**) possesses a lanostane skeleton. Comparison of the structure of compound **1**, in a retrosynthetic sense, with the structure of lanosterol (**2**), a commercial product from the wool industry, reveals that **1** may be readily available from **2**. This transformation might be achieved by oxidative cleavage of ring A in lanosterol to generate a 3-carboxylic acid and 4 (28)-alkene, and selective oxidation of the terminal allylic methyl of the side chain to an α,β-unsaturated carboxyl group with a Z-configuration.

Several attempts to stereoselectively transform the allylic methyl of compound **2** directly to a (Z)-allylic alcohol or (Z)-carbonyl were unsuccessful. In contrast, treatment of lanosterol (**2**) with selenium dioxide stereoselectively oxidized a terminal allylic methyl of lanosterol to give the (E) α,β-unsaturated aldehyde in high yield. Attempts to transform the (E) aldehyde to the Z-isomer also failed. Therefore, a less direct route to the (Z) α,β-unsaturated acid through oxidative cleavage of the double bond of the side chain to an aldehyde(**4**) followed

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by a stereoselective Wittig reaction to introduce an (Z) α,β-unsaturated hydroxymethyl group was employed.

The C-3 hydroxyl of lanosterol (**2**) was protected, and the resultant tetrahydropyranyl (THP) ether derivative **3** was ozonized in pyridine at -78° C to afford compound **4**. Compound **4** was subjected to a modified Wittig reaction according to the procedures of Corey[2] and Schlosser,[3] yielding the allylic alcohol **5** with a Z / E ratio of 19:1 in 54% yield. Characterization of compound **5** by ^{1}H NMR reveals that the olefinic H-24 resonates at δ 5.10 in the predominant Z isomer, while in the minor E isomer H-24 is located downfield at δ 5.40. Compound **5** was quantitatively oxidized to aldehyde **6** with fresh MnO_2. Further oxidation with sodium chlorite[4] gave a 90% yield of carboxylic acid **7**. Removal of the THP protection group in compound **7** furnished compound **8**, which gave anwuweizonic acid (**9**, 92% yield) on pyridinium chlorochromate (PCC) oxidation. Each of compounds **8** and **9** are known; the latter was isolated from the same plant material as compound **1**.[1]

Cleavage of ring A to convert compound **9** into manwuweizic acid (**1**) had been achieved by employing a series of transformations described in our earlier paper:[1] oximation of anwuweizonic acid (**9**) followed by an abnormal Beckmann rearrangement with *p*-tosyl chloride in pyridine afforded 3,4-secolanosta-4(28), 8,24Z-trien-3-cyano-26-oic acid (**12**) in 25% yield. Hydrolysis of the nitrile group of compound **12** provided manwuweizic acid (**1**).

In above abnormal Beckmann rearrangement of **10**, the nitrile **12** is a minor product while the major product is a lactam. In order to improve the yield of nitrile compound **12**, some reaction conditions were modified. We found that the use of DMSO-DCC-TFA changed the nitrile / lactam ratio to favor the nitrile compound, presumably because DMSO reacts with the unprotected carboxyl group forming a methylthiomethyl (MTM) ester prior to rearrangement. Thus, compound **10**, on treatment with DMSO-DCC-TFA, gave a mixture of nitrile **11** in 53% yield and lactam **13** in 41% yield. Alkaline hydrolysis of compound **11** afforded compound **1** in 91% yield, identical with an authentic sample of natural manwuweizic acid.

A study of the hydrolysis reaction of compound **11** showed that hydrolysis of the MTM ester occurs prior to hydrolysis of the cyano group and that compound **12** is an intermediate in the hydrolysis. The reagent of DMSO-DCC-TFA provides a convenient method for protection of a carboxyl group. We also examined the reaction of benzoic, cinnamic, tiglic and myristic acids with DMSO-DCC-TFA. In the presence of one equivalent of TFA, two equivalents of DCC and excess of DMSO, an MTM ester forms easily at room temperature, in yields over 90%. The cleavage of the MTM group under mild condition was already reported in literature.[5,6]

Experimental. Mps: uncorrected. Silica gel: Quingdao Haiyang Chemical Factory, 200-300 mesh. HPTLC plates: Yantai Institute of Chemical Technology. The following instruments were used to record spectra: IR, Perkin Elmer 599B with KBr as the medium; ^{1}H NMR, Bruker AM 400 (400 MHz) and JEOL PS-100 (100 MHz); HRMS, Varian MAT-711.

3β-Tetrahydropyranyl lanosterol (3) and 3β-tetrahydropyranyloxy-25,26,27-trinor-lanosta-8-en-24-al (4).

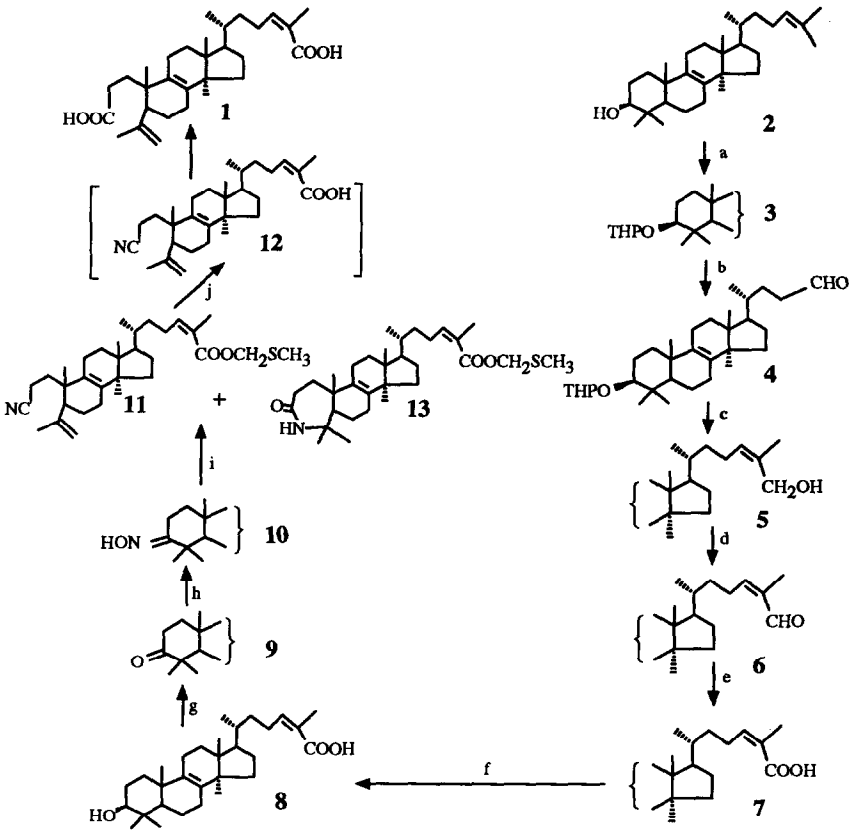

Chart 1

a. DHP, PPTS; b. O_3, Py, -78°/ CH_3SCH_3; c. Ph_3PEtBr, nBuLi, -78°/ nBuLi, HCHO, THF, 0°; d. MnO_2, hexane; e. $NaClO_2$, NaH_2PO_4, β-isoamylene, t-BuOH; f. PPTS, EtOH; g. PCC, CH_2Cl_2; h. $NH_2OH.HCl$, Py; i. DCC, DMSO, TFA; j. KOH, EtOH.

Pyridinium *p*-toluenesulfonate (PPTS) (502 mg, 2.0 mmol) and 3,4-dihydro-2H-pyran (DHP) (4.0 mL, 44.0 mmol) were added to a stirred solution of **2** (8.50 g, containing 40-50% dihydrolanosterol, ~20.0 mmol) in CH_2Cl_2(100 mL). The mixture was stirred for 5 h at r.t., then filtered. The filtrate was passed through a Si gel column (10 x 10 cm); the gel was washed with petrol to give **3** and its dihydroderivative (9.25 g, 91%) as a white solid.The reaction product (9.20 g, ~9.5 mmol of **3**) in CH_2Cl_2 (500 mL) and pyridine (5 mL) was treated with ozone at -78° C for 20 min. Argon was bubbled through the reaction mixture for 5 min, and then dimethyl sulfide (5 mL, 6.8 mmol) was added. The reaction mixture was stirred at r.t. overnight, and then concentrated. The residue was purified by CC over Si gel (5 x 25 cm) eluting with petrol and petrol-ether (10:1). The fractions eluted by petrol-ether (10:1) were crystallized from methanol to afford compound **4** (4.1 g, 86%). Mp.84-86° C, ^{1}H NMR ($CDCl_3$, 100 MHz) δ: 9.68 (1H,s, CHO), 4.50, 4.70 (0.5 H each, m, H-1 of THP), 3.90 (1H, m, H-3α), 0.80 (3H, d, J=6Hz, H-21), 0.93, 0.87, 0.82, 0.81, 0.64 (3H each, s, t-CH_3 x 5) ppm; MS m/z: 484 [M^+], 469, 456, 441, 400[M-C_5H_8O]$^+$, 385; IR ν max: 2815, 2710, 1730 cm^{-1}.

3β-Tetrahydropyranyloxy-lanosta- 8,24Z-dien-26-ol (5). n-Butyllithium (5.0 mL of 1.6 mol solution in hexane, 8.0 mmol) was added to a cooled (0° C) and stirred suspension of ethyltriphenylphosphonium bromide (3.018 g, 8.0 mmol) in dry THF (20 mL) under an argon atmosphere. The red reaction mixture was stirred for 10 min, then cooled to -78° C. Aldehyde **4** (3.380 g, 7.0 mmol) in dry THF (20 mL) was added dropwise and the reaction mixture faded to orange-yellow. Another portion of n-butyllithium (4.5 mL, 7.3 mmol) was added dropwise and the reaction mixture changed to deep red. After further stirring for 10 min at -78° C, the temperature of the mixture was raised to 0° C, and a freshly prepared solution of formaldehyde* in THF (30 mL) was added rapidly by syringe. The red color of mixture faded immediately. After being stirred for 30 min at 0° C, the reaction was quenched with brine and extracted with ether. The residue of the extract was chromatographed over Si gel (3 x 20 cm) eluting with petrol-ether (10:1). The major product was crystallized from petrol to give **5** (1.98 g, 54%). Mp. 79-81° C, ^{1}H NMR (100 MHz, CCl_4) δ: 5.10 (1H,t,J=7Hz,H-24), 4.50, 4.65 (0.5H each,m,H-1 of THP), 3.92 (2H,s,H-26), 3.84 (1H,m,H-3α), 1.72 (3H,s,H-27), 0.86 (3H,d,J=6Hz,H-21), 0.97, 0.84, 0.79, 0.66 (6H,3H,3H,3H each,s,t-CH_3 x 5) ppm; HRMS m/z (rel. int.): 526.4372 [M^+, calcd for $C_{35}H_{58}O_3$, 526.4371](3), 511.4165 [M-Me]$^+$ (3), 427.3548 [M-Me-C_5H_8O]$^+$ (4), 409.3460 [M-Me-C_5H_8O-H_2O]$^+$(10), 391.3395 [M-Me-C_5H_8O-2 H_2O]$^+$(4); IR ν max: 3400, 1450, 1370cm^{-1}.

*A solution of formaldehyde was generated by dropwise addition of boron trifluoride etherate (0.20 mL, 1.6 mmol) to a solution of paraformaldehyde (1.50 g) in THF under argon at such rate that the formaldehyde slowly distilled. The distillate was stored at -78° C.

3β-Tetrahydropyranyloxy-lanosta-8,24Z-dien-26-al (6). Freshly prepared active MnO_2[7](2.50 g, 28.7 mmol) was added to the solution of **5** (750 mg, 1.43 mmol) in hexane (20 mL) and the mixture was stirred for 45 min at r.t. The suspension was filtered through a short column of Si gel and washed with ether. The combined organic fractions were concentrated to afford crystals of **6** (730 mg, 98%). Mp. 92-94° C, ^{1}H NMR (100 MHz, CCl_4) δ: 9.94 (1H,s,CHO), 6.25 (1H,t,J=7Hz, H-24), 4.40, 4.60 (0.5H each,m,H-1 of THP), 3.76 (1H,m,H-3α), 2.50-2.28 (2H,m,H-23), 1.66 (3H,s,H-27), 0.86 (3H,d,J=6Hz,H-21), 0.92, 0.80, 0.72, 0.62 (6H,3H,3H,3H each,s,t-CH_3 x5) ppm; MS m/z: 524 [M^+], 509 [M-CH_3]$^+$, 440[M-C_5H_8O]$^+$, 425[M-CH_3-C_5H_8O]$^+$, 407 [M-CH_3-C_5H_8O-H_2O]$^+$; IR ν max: 2720, 1670 cm^{-1}.

3β-Tetrahydropyranyloxy-lanosta-8,24Z-dien-26-oic acid (7). An aqueous solution (20 mL) of $NaClO_2$ (4 g, 44.2 mmol) and NaH_2PO_4 (3 g, 25.0 mmol) was added slowly to a solution of **6** (710 mg, 1.35 mmol) and β-isoamylene (6 mL, 56.6 mmol) in t-butyl alcohol (25 mL). The mixture was stirred vigorously for 16 h at r.t., then diluted with water (70 mL), and extracted with ether. The residue of the extract was purified by CC over Si gel (2 x 20 cm) eluting with petrol-ether (4:1) to give **7** (650 mg, 89%) crystallized from ether-petrol (1:1). Mp. 154-155° C, ^1H NMR (100 MHz, $CDCl_3$) δ: 6.01 (1H,t,J=7Hz,H-24), 4.70, 4.45 (0.5H each,m,H-1 of THP), 3.88 (1H,m,H-3α), 2.58-2.26 (2H,m,H-23), 1.83 (3H,s,H-27), 0.88 (3H,d,J=6Hz,H-21), 0.94, 0.82, 0.78, 0.64 (6H,3H,3H,3H each,s,t-CH_3 x 5) ppm; HRMS m/z (rel. int.): 540.4139 [M^+, calcd for $C_{35}H_{56}O_4$, 540.4164] (11), 525.4052 [M-Me]$^+$ (9), 456.3588 [M-C_5H_8O]$^+$ (7), 441.3389 [M-Me-C_5H_8O]$^+$ (31), 423.3215 [M-Me-C_5H_8O-H_2O]$^+$ (70); IR ν max: 3500-2500, 1695 cm^{-1}.

3β-Hydroxy-lanosta-8,24Z-dien-26-oic acid (8). PPTS (25 mg, 0.10 mmol) was added to a stirred solution of **7** (600 mg, 1.11 mmol) in ethanol (10 mL). The mixture was heated at 55° C for 4 h, then concentrated. The residue was purified by Si gel column (2 x 5 cm) eluting with ether. Compound **8** (502 mg, 99%) crystallized from methanol, mp. 145-147° C. ^1H NMR (400 MHz, $CDCl_3$) δ: 6.06 (1H,t,J=6.8Hz,H-24), 3.22 (1H,dd,J=4.6/11.5Hz,H-3α), 2.54, 2.43 (1H each,m,H-23), 1.90 (3H,s,H-27), 0.90 (3H,d,J=6.4Hz,H-21), 0.98, 0.96, 0.85, 0.79, 0.67 (3H each,s,t-CH_3 x 5) ppm; HRMS m/z (rel. int.): 456.3635 [M^+, calcd for $C_{30}H_{48}O_3$, 456.3591] (16), 441.3323 [M-Me]$^+$ (70), 423.3204 [M-Me-H_2O]$^+$ (100), 301.2135 [$C_{20}H_{29}O_2$]$^+$ (15), 287.1989 [$C_{19}H_{27}O_2$]$^+$ (11), 241.1918 [$C_{18}H_{25}$]$^+$ (16); IR is identical with the authentic sample.[1]

Anwuweizonic acid (9). PCC (1.10 g, 5.10 mmol) was added to a solution of **8** (500 mg, 1.10 mmol) in dichloromethane (30 mL) and stirred for 3 h at r.t. The reaction mixture was filtered through a short column of Si gel (2 x 4 cm) and eluted with ether. Compound **9** (460 mg, 92%) crystallized from acetone-petrol is identical with a natural sample of anwuweizonic acid (mmp, IR, ^1H NMR, HRMS and $[\alpha]_D$).[1]

Lanosta-8,24Z-dien-3-isonitroso-26-oic acid (10). Hydroxylamine hydrochloride (70 mg, 1.0 mmol) was added to a solution of **9** (430 mg, 0.95 mmol) in ethanol (5 mL) and pyridine (5 mL). The mixture was refluxed for 2 h and then the solvent was removed *in vacuum*, and water (20 mL) was added to give a white solid. Crystallization from methanol provided **10** (412 mg, 93%), mp. 184-186° C. ^1H NMR (100 MHz, $CDCl_3$) δ: 9.90 (2H,brs,COOH and NOH), 6.02 (1H,t,J=7 Hz, H-24), 1.88 (3H,s,H-27), 1.12, 1.07, 0.80, 0.65 (3H,6H,3H,3H each,s,t-CH_3 x 5), 0.86 (3H,d,J=6 Hz,H-21) ppm; MS m/z: 469 [$M]^+$, 454 [M-Me]$^+$, 436, 418; IR ν max: 3600-2300, 3260, 1690, 1640 cm^{-1}; Anal. Calcd for $C_{30}H_{47}NO_3$: C, 76.71, H, 10.09, N, 2.98. Found: C, 76.74, H, 10.23, N, 3.18.

Methylthiomethyl 3,4-seco-lanosta-4(28),8,24Z-trien-3-cyano-26-oate (11) and methylthiomethyl A-homo-4-azalanosta-8,24Z-dien-3-one-26-oate (13). Trifluoroacetic acid (TFA) (0.60 mL 7.79 mmol) and 1,3-dicyclohexylcarbodiimide (DCC) (1.50 g, 7.27 mmol) were added to a stirred and cooled (0°) solution of **10** (1.00 g, 2.13 mmol) in benzene (15 mL) and DMSO (10 mL). The mixture was stirred for 15 min and another portion of DCC (0.75 g, 3.63 mmol) was added to the mixture. Reaction was then allowed to proceed for 3 h at 70° C. The mixture was cooled to 0° C and ether (30 mL) was added to precipitate 1,3-dicyclohexylurea. The

precipitate was filtered off and the filtrate was washed with water and concentrated. The residue was chromatographed over Si gel (3 x 25 cm) eluting with cyclohexane-acetone (20:1, 5:1). Compound **11** (582 mg, 53%) was obtained from the early fractions and compound **13** (463 mg, 41%) from the late fractions. Compound **11** is amorphous: ^{1}H NMR (100 MHz, $CDCl_3$) δ: 5.96 (1H,t,J=7Hz,H-24), 5.20 (2H,s,OCH_2S), 4.91, 4.63 (1H each,s, $C=CH_2$), 2.22 (3H,s,SCH_3), 1.86 (3H,s,H-27), 1.70 (3H,s,H-29), 0.90 (3H,d,J=6Hz,H-21), 0.92, 0.88, 0.68 (3H each,s,t-CH_3 x 3) ppm; HRMS m/z (rel. int.): 511.3469 [M^+, calcd for $C_{32}H_{49}NO_2S$, 511.3472] (27), 496.3299 [M-Me]$^+$ (15), 457.3152 [M-CH_2CH_2CN]$^+$ (7); IR ν max: 2240(CN), 1720, 1635, 1310 cm^{-1}; Anal. Calcd for $C_{32}H_{49}NO_2S$: C, 75.09, H, 9.65, N, 2.74, S, 6.27. Found: C, 74.72, H, 9.72, N, 2.68, S, 6.40. Compound **13** is amorphous: ^{1}H NMR (100 MHz, $CDCl_3$) δ: 7.28 (1H,brs,NH), 5.92 (1H,t,J=7Hz,H-24), 5.16(2H,s,OCH_2S), 2.20 (3H,s,SCH_3), 1.86 (3H,s,H-27), 1.28, 1.21, 1.14, 0.84,0.66 (3H each,s,t-CH_3 x 5), 0.88 (3H, covered, H-21) ppm; MS m/z: 530[M+1]$^+$, 529 [M^+], 515, 514 [M-Me]$^+$, 467; IR ν max: 3210, 3070, 1720, 1675, 1650 cm^{-1}.

Mawuweizic acid (1). A 40% aqueous solution (3 mL) of KOH was added to a solution of **11** (120 mg, 0.235 mmol) in ethanol (5 mL) and refluxed for 24 h. After the ethanol had been removed, the aqueous solution was acidified to pH 4-5 with 6N HCl, and then extracted with ether. The ether extract was washed with brine, dried (Na_2SO_4), and concentrated. Crystallization from methanol gave **1** (100 mg, 91%) identical with a natural sample of mawuweizic acid (mmp, IR, ^{1}H NMR, HRMS).[1]

Methylthiomethyl esterification of carboxylic acid. General procedure for benzoic, cinnamic, tiglic and myristic acids: TFA (0.10 mL) and DCC (2.0 mmol) were added with stirring at r.t. to a solution of the carboxylic acid (1.0 mmol) in benzene (2 mL) and DMSO (0.5 mL). Solid DCC immediately dissolved exothermically and 1,3-dicyclohexylurea precipitated. The mixture was allowed to stand for 10 min. Ether (20 mL) was added and allowed to stand for 1 h to precipitate additional 1,3-dicyclohexylurea. The precipitate was filtered off, and then the filtrate was washed with water and dried (Na_2SO_4). The reaction product was purified by CC over Si gel (2 x 10 cm) using petrol-ether (20:1) to give pure MTM esters (yield over 90%).

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References. 1 Liu,J-S.; Huang,M-F.; Tao,Y. *Can. J. Chem.*, **1988**, 66, 414-415.
2. Corey,E.J.; Yamamoto,H. *J. Am. Chem. Soc.*, **1970**, 92, 226-228.
3. Schlosser,M.; Coffinet,D. *Synthesis*, **1971**, 380-381.
4. Bal,B.S.; Childers,W.E.Jr.; Pinnick,H.W. *Tetrahedron*, **1981**, 37, 2091-2096.
5. Wade,L.G.; Gerdes,J.M.; Wirth,R.P. *Tetrahedron Lett.*, **1978**, 731-732.
6. Ho,T-L.; Wong,C.M. *J. Chem. Soc., Chem. Commun.* **1973**, 224-225.
7. Attenburrow,J. et al. *J. Chem. Soc.*, **1952**, 1094-1111.