Nigranoic Acid, a Triterpenoid from *Schisandra sphaerandra* That Inhibits HIV-1 Reverse Transcriptase

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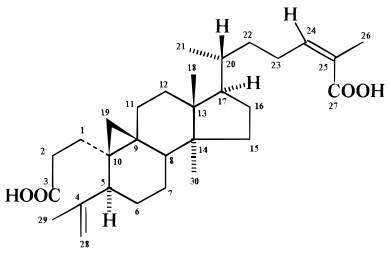
Received December 27, 1995[⊗]

An A ring-secocycloartene triterpenoid, nigranoic acid (3,4-secocycloarta-4(28),24-(*Z*)-diene-3,-26-dioic acid, (**1**) was isolated from the stems of *Schisandra sphaerandra*, a Chinese traditional medicinal plant. Its structure elucidation and unambiguous NMR spectral assignment were achieved by the combination of 1D- and 2D-NMR techniques with the aid of computer modeling. Nigranoic acid showed activity in several anti-HIV reverse transcriptase and polymerase assays.

Schisandra sphaerandra Stapf. (Schisandraceae), distributed in Southern China, is a traditional medicinal plant that has been used for the treatment of stomach disorders.[1] In our continuing search for biologically active constituents of Chinese medicinal plants, nigranoic acid (**1**), an anti-HIV RT triterpenoid, was isolated from the petroleum ether extract of the stems based on bioassay-guided fractionation, and we report here on its isolation, structure elucidation, and bioassay.



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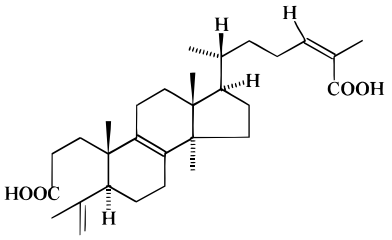
The molecular formula $C_{30}H_{46}O_4$ of nigranoic acid (**1**) was established by high-resolution mass measurement. In the carbonyl absorption region, the IR spectrum displayed two carbonyl bands, one of them indicative of a carboxylic acid (1705, 3050−2870 cm^{-1}) and the second band at 1690 cm^{-1} of an α,β-unsaturated carbonyl group, and these inferences were supported by the ^{13}C-NMR spectrum (δ 176.54 and 170.90, respectively). The ^1H-NMR showed the presence of two olefinic methyl groups (δ 2.08, 1.67), one isopropenyl group (methylene protons at δ 4.79 and 4.94, 28a-H and 28b-H; methyl group at δ 1.67, 29-CH_3), and an angelic acid moiety (δ 6.00, t, J = 7.0 Hz, 24-H; δ 2.08, 26-CH_3). Two tertiary methyl groups (δ 0.87 and 0.90) and one secondary methyl group at δ 0.95 (d, J = 6.0 Hz, 21-CH_3) were also observed, which suggested the presence of a 3,4-*seco*-ring A triterpenoid possessing the same side chain as that present in manwuweizic acid (**2**).[2]



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The presence of a cycloartane methylene group instead of a 19-CH_3 and an 8,9-double bond in **2** were deduced from its ^1H-NMR spectrum. Compound **1** possessed the same MF as **2**, but there was a significant high-field, nonequivalent methylene couplet at δ 0.63 and 0.34 in **1**, with the disappearance of both a methyl group and a double bond compared with **2**. Therefore, the structure of **1** was suggested to be 3,4-secocycloarta-4(28),24(*Z*)-diene-3,26-dioic acid. This compound was previously named nigranoic acid and was isolated from *Schisandra nigra* by saponifying the oily methyl nigranoate, itself obtained by the separation of the esterified crude extract.[3] No detailed spectroscopic data in support of the structure were available, and this is the first report on the isolation of this compound as a free diacid. It is a significant challenge to assign the NMR resonances of **1** because of the severely overlapping signals, even in the high-field spectra. These difficulties were resolved by taking advantage of various 1D- and 2D-NMR techniques including DEPT, COSY, HETCOR, selective INEPT, HOHAHA, and ROESY and molecular modeling experiments.

The proton signals of 19-H (α, β), 24-H, 21-CH_3, 27-CH_3, and 29-CH_3 could be assigned by taking into account the chemical shifts and coupling pattern by means of COSY and ROESY experiments, which resulted in the assignment of their corresponding carbon-13 resonances by HETCOR (Table 1).

The methine protons of 5-H at δ 2.48 and 8-H at δ 2.43 could be readily deduced by the observation of scalar peaks with 28b-H at δ 4.94 and 19b-H at δ 0.63, respectively, in the ROESY spectrum. Consequently, C-5 and C-8 were assigned to δ 46.30 and 48.16 from the HETCOR experiment. The two remaining coupled

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[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1996.

S0163-3864(96)00149-8 CCC: $12.00

Table 1. NMR Assignments of Nigranoic Acid (**1**)[a]

	^{13}C-NMR	^{1}H-NMR		^{13}C-NMR	^{1}H-NMR
1	30.29 t	2.78 (m); 2.47 (m)	16	28.72 t	1.59 (m); 1.22 (m)
2	32.85 t	2.32 (m); 1.57 (m)	17	52.79 d	1.57 (m)
3	176.54 s		18	18.67 q	0.90 (s)
4	150.32 s		19	30.46 t	0.63 (d, J = 4.5 Hz, β); 0.34 (d, J = 4.5 Hz, α)
5	46.30 d	2.48 (m)	20	36.73	1.59 (m)
6	28.29 t	0.98 (m); 1.51 (m)	21	18.77 q	0.95 (d, 6.0 Hz)
7	25.59 t	0.81 (m); 1.16 (m)	22	27.54 t	1.72 (m); 1.42 (m)
8	48.16 d	1.43 (m)	23	27.42 t	2.81 (m); 2.75 (m)
9	21.88 s		24	143.04 d	6.00 (t, J = 7.0 Hz)
10	27.91 s		25	128.91 s	
11	36.73 t	1.91 (m); 1.62 (m)	26	21.92 q	2.08 (s)
12	36.22 t	1.74 (m); 1.56 (m)	27	170.90 s	
13	45.69 s		28	112.20 t	4.94 (br s, b); 4.79 (br s, a)
14	49.51 s		29	20.41 q	1.67 (s)
15	33.63 t	1.88 (m)	30	19.85 q	0.87 (s)

[a] ppm from internal standard TMS in pyridine-d_5.

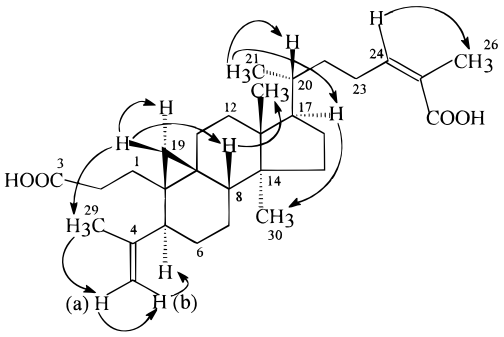

Figure 1. ROE correlations of nigranoic acid (**1**) by the ROESY spectrum.

methine protons at δ 1.57 and 1.59 could be assigned to 17-H and 20-H, respectively, because the latter showed a scalar connection with the doublet 21-CH_3 signal at δ 0.95. The methyl signals at δ 0.90 and 0.87 were assigned to 18-CH_3 and 30-CH_3 based on ROE's with the 8-H and 17-H, respectively.

Computer modeling was carried out with the PC-MODEL program (Serena Software, 1993). From the energy-minimized conformation, the spatial near approach between 19b-H/29-CH_3 (2.27 Å), 19b-H/8-H (2.73 Å), 17-H/30-CH_3 (2.10 Å), and 8-H/18-CH_3 (2.34 Å) was consistent with the indicated ROE cross peaks observed between the above proton pairs in the ROESY spectrum (Figure 1).

The methylene protons at δ 2.78 and 2.47, assigned to 2-H, were scalar coupled with the methylene signals at δ 2.32 and 1.59, which, in turn, showed long-range correlations with the resonance at δ 176.54 and thus could be assigned to 1-H. Moreover, the 5-H was scalar coupled to the resonances at δ 0.98 and 1.51, assigned to 6-H, and was long-range coupled to the methylene protons in the HOHAHA spectrum at δ 0.81 and 1.16, assigned to 7-H. This assignment was confirmed by the observation of coupling correlations of 8-H to both 7-H and 6-H. Meanwhile, 11-H, 12-H, 15-H, 16-H, 22-H, and 23-H could be assigned in a similar way as described above. Long-range ^{1}H−^{13}C-NMR correlations, determined using the selective INEPT technique, allowed for the assignment of the quaternary carbon signals at C-4, C-9, C-10, C-13, C-14, and C-25, as shown in Table 1.

Nigranoic acid was found to be active in several reverse transcriptase and polymerase assays, in which fagaronine chloride (IC_{50} 10 μg/mL) was adopted as a positive-control substance, and the results are shown in Tables 2 and 3.

Since reverse transcriptase (RT) is required for early proviral DNA synthesis,[4] inhibition of the RT-catalyzed polymerization of DNA from viral RNA inhibits virus replication. It is also logical that RTs may be virus specific and thus are considered viable chemotherapeutic targets. Although nucleoside analogues, such as azidothymidine (AZT), which is converted to triphosphate by cellular enzymes and acts as a chain terminator,[5] have been approved for clinical use in HIV-1 infection, there are substantial toxic side effects associated with their use,[6] and complete inhibition of viral replication is not achieved.[7] Moreover, the emergence of nucleotide-resistant HIV strains may complicate long-term therapy.[8] Hence, the discovery and characterization of new agents capable of specifically inhibiting HIV RT without mediating a toxic response is urgent.

Natural products serve as one source of structurally novel chemicals and are expected to be fruitful for investigation as specific inhibitors of HIV RT. We have developed a bioassay for the screening of natural products and plant extracts for their HIV-1 RT inhibitory potential, which has led to the finding of some active isolates through bioassay-guided fractionation discovery.[9,10] Among them, 1β-hydroxyaleuritolic acid 3-*p*-hydroxybenzoate, a triterpenoid acid derivative, was assumed to be a mixed-type competitive inhibitor with respect to the template-primer and a noncompetitive inhibitor with respect to substrate (TTP) and has no specific effect on the substrate binding site (nucleotide binding site) or the template-primer binding site.[11] As demonstrated here, the isolation of **1**, a triterpenoid dioic acid, represents another example of a natural product that inhibits HIV-1 RT. That **1** was isolated from a Chinese traditional medicinal plant *S. sphaerandra*, which has been safely used since ancient times and which appears to be nontoxic, implies that a continued search for leads for AIDS chemotherapy from higher plants may indeed be promising.

Experimental Section

General Experimental Procedures. Melting point is uncorrected. IR spectrum was recorded in a KBr pellet on a MIDAC FT-IR interferometer. The optical rotation was measured with a Perkin-Elmer 241 polarimeter. The ^{1}H- and ^{13}C-NMR, COSY, HETCOR, HOHAHA, and ROESY spectra were recorded at 500.12 MHz for ^{1}H and 125.76 MHz for ^{13}C with a GE OMEGA 500 instrument using GE standard programs in C_5D_5N

Table 2. Results of Reverse Transcriptase and Polymerase Assays with Nigranoic Acid (**1**)[a]

% inhibition at 200 μg/mL [IC_{50}, μg/mL, μM]						
HIV-1 RT	HIV-2 RT	Mutant RT	AMV RT	DNA pol. α	DNA pol. β	RNA pol.
99.4	76.7	71.0	99.2	46.2	99.1	75.4
[74.1; 159.4]	[167.8; 356.5]	[182.7; 388.1]	[113.7; 241.6]	[>200; 424.9]	[7.8; 16.6]	[88.3; 187.6]
MA	WA	WA	MA	IA	A	MA

[a] BSA = 250 μg/mL.

Table 3. Results of Differential-HIV-1 RT Activity with Nigranoic Acid (**1**)[a]

% inhibition at 200 μg/mL [IC_{50}, μg/mL, μM]		
DDDP	RDDP	RNAase H
96.7	99.4	6.8
[19.1; 40.6]	[74.1; 157.4]	[>200; 424.9]
A	MA	IA

[a] Key: A = active, MA = moderately active, WA = weakly active, IA = inactive, DDDP = DNA-dependent DNA polymerase activity; RDDP = RNA-dependent DNA polymerase activity.

solution. DEPT and selective INEPT experiments were recorded at 90.8 MHz with a Nicolet NMC-360 instrument. For the selective INEPT experiments, data sets of 16K covering a spectral width of 10 kHz were acquired. Proton pulse widths were calibrated using a sample of HOAc in 10% C_6D_6 (^{1r}J = 6.7 Hz) in a 5-mm NMR tube.[12] Electron impact mass spectra (70 eV) were recorded with a Varian MAT-112S mass spectrometer, and high-resolution mass spectra were recorded with a Finnigan MAT-90 instrument.

Plant Material. The stems of *S. sphaerandra* were collected in Yunnan Province, People's Republic of China, and identified by Prof. H. W. Li of the Kunming Institute of Botany, where a voucher specimen is deposited.

Extraction and Isolation. The dried powdered stems of *S. sphaerandra* (521 g) were extracted with petroleum ether, and white needles were obtained after evaporation of the extracts under vacuum. Nigranoic acid (13.2 g, yield 2.5%) was afforded as fine needles after recrystallization using petroleum ether (bp 30−60 °C).

Nigranoic Acid (1). White needles; mp 128−130 °C, $[\alpha]_D$ +61.5° (*c* 1.15, MeOH) [lit.[3] $[\alpha]_D$ +55.8° (*c* 2.4, MeOH)]: IR λ_{max} (cm^{-1}) 3050−2870 (s), 2600, 1710, 1690, 1640, 1455, 1413, 1374, 1257, 1218, 1163, 1077, 933, 890; MF $C_{30}H_{46}O_4$ (HRMS 470.3393, calcd 470.3396); EIMS *m*/*z* (70 eV) 470 (M^+, 15), 455 (45), 397 (20), 371 (18), 329 (23), 235 (30), 150 (35), 121 (52), 107 (70), 95 (100), 55 (73). ^1H and ^{13}C-NMR data are shown in Table 1.

HIV-1 RT Inhibition Assays. The biological evaluations for HIV-1 RT inhibition and polymerase activities of nigranoic acid were carried out according to established protocols.[9,10]

Acknowledgment. This work was supported, in part, by grants from Kunming Institute of Botany, Academia Sinica, People's Republic of China, to the Chinese research group and a grant from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, to the U.S. research group. The authors thank Prof. H.-W. Li for the identification of the plant materials, Mr. R. Dvorak for MS, and the Research Resources Center of the University of Illinois at Chicago for the provision of NMR spectroscopic facilities.

References and Notes

(1) Jiangsu College of New Medicine. *Dictionary of Chinese Traditional Medicine*; Shanghai Science and Technology Press: Shanghai, 1986; pp 1859−1860.
(2) Liu, J.-S.; Huang, M.-F.; Tao, Y. *Can. J. Chem*. **1988**, *66*, 414−415.
(3) Kikuchi, M.; Yoshikoshi, A. *Chem. Lett*. **1972**, 725−728.
(4) Sarin, P. S. *Ann. Rev. Pharmacol. Toxicol*. **1988**, *28*, 411−428.
(5) Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St. Clair, M. H.; Nusinoff-Lehrman, S.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A*. **1985**, *82*, 7096−7100.
(6) Johnson, M. I.; Hoth, D. F. *Science* **1993**, *260*, 12861293.
(7) Ho, D. D.; Moudgil, T.; Alam, M. *N. Engl. J. Med*. **1989**, *321*, 1621−1625.
(8) Larder, B. A.; Darby, G.; Richman, D. D. *Science* **1989**, *243*, 1731−1734.
(9) Tan, G. T.; Pezzuto, J. M.; Kinghorn, A. D.; Hughes, S. H. *J. Nat. Prod*. **1991**, *54*, 143−154.
(10) Tan, G. T.; Pezzuto, J. M.; Kinghorn, A. D. In *Natural Products as Antiviral Agents*; Chu, C. K., Gutler, H. G., Eds.; Plenum Press: New York, 1992; pp 195−222.
(11) Pengsuparp, T.; Cai, L. N.; Constant, H.; Fong, H. H. S.; Lin, L. Z.; Kinghorn, A. D.; Pezzuto, J. M.; Cordell, G. A.; Ingolfsdottir, K.; Wagner, H.; Hughes, S. H. *J. Nat. Prod*. **1995**, *58*, 1024−1031.
(12) Bax, A. *J. Magn. Reson*. **1983**, *52*, 76−80.

NP960149H