Journal of Natural Products
Vol. 49, No. 6, pp. 1114-1116, Nov-Dec 1986

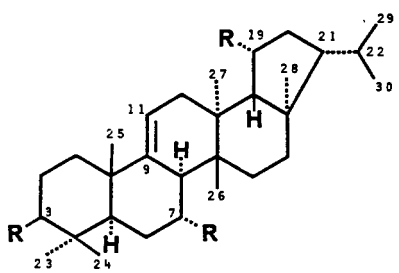
RUBIATRIOL, A NEW TRITERPENOID FROM THE CHINESE DRUG, "QIÁN CÁO GÉN," *RUBIA CORDIFOLIA*[1]

MUNEHISA ARISAWA,* HAJIME UENO, MASAYUKI NIMURA, TOSHIMITSU HAYASHI, and NAOKATA MORITA

Department of Medicinal Resources, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan

The Chinese crude drug "Qián Cáo Gén," as described in the dictionary of Chinese Crude Drugs (Zhong-yao-da-cidian) (1), is the dried root or rhizome of *Rubia cordifolia* L. (Rubiaceae) or its varieties *longifolia* Hand.-Mazz. and *pratensis* Maxim., *Rubia chinensis* Reg et Maack, and *Rubia truppeliana* Loes. The crude drug has already afforded anthraquinones (2-6), flavonoids (7,8), iridoid glycosides (9), and miscellaneous compounds (4,5,7,9,10). Recently, several cytotoxic cyclic hexapeptides were isolated from the drug, and their structures were confirmed by the Itokawa group (11).

As a part of a program of studies on biologically active constituents from natural resources, we have obtained a new triterpenoid, named rubiatriol (**1**), and two known anthraquinones, 2-methyl-1,3,6-trihydroxyanthraquinone and alizarin, from the $CHCl_3$-soluble fraction of the MeOH extract, which has inhibitory activity on the angiotensin converting enzyme (ACE) (12).



1 R=OH
2 R=OAc
3 R=H

Rubiatriol (**1**) showed a positive Liebermann-Burchard (LB) reaction, and hydroxy (3360 cm^{-1}) and double bond (1630 cm^{-1}) absorptions were observed in its ir spectrum. The ms spectrum of **1** showed a molecular ion peak at *m/z* 458 and the prominent peaks for a pentacyclic triterpene alcohol having a double bond at the 8 or 9 (11)-position (13). The ^{1}H-nmr spectrum of **1** showed signals for six singlet angular methyl groups, two doublet methyl groups of an isopropyl group, three multiplet methine groups bearing hydroxy groups, and an olefinic proton. The ^{13}C-nmr spectrum also suggested three carbons (δ 71.40, 74.52, and 75.14) bearing hydroxy groups and two olefinic carbons (δ 116.98 and 121.11). The signals of two doublet methyls at δ 0.82 and 0.88, a singlet methyl at δ 1.06, and an olefinic proton at δ 5.31, suggested a fern-9 (11)-ene nucleus (14). The singlet methyls at δ 0.80 and δ 0.99 and a methine proton at δ 3.20 were assignable to the protons of the 24, 23, and 3α-positions, respectively (14,15). The methyl signals at δ 0.92 and 0.98 were shifted downfield by 0.16 from those of 28-H_3 and 27-H_3 of fern-9(11)-ene (**3**), a lesser shift was observed for 26-H_3 (0.10). These data suggested that the remaining two hydroxy groups are present at the 7- and 19-positions. Acetylation of **1** afforded a triacetate (**2**) as colorless needles. The ^{1}H nmr of **2** showed three methine proton signals at δ 4.46, 4.97, and 5.07. The signal at δ 4.46 (dd, J=4.2 and 10.9 Hz) is assignable to 3α-H. The two sextet signals at δ 4.97 (J=5.5, 10.3, and 10.3 Hz) and 5.07 (J=3.5, 9.5, and 9.5 Hz) are assignable

[1]These data were first presented at the 65th Meeting of the Hokuriku Branch of the Pharmaceutical Society of Japan, Toyama, June, 1985.

to the protons located at 7β and 19β, respectively. From these spectral data, the structure of rubiatriol (**1**) is proposed to be 3β, 7α, 19α-trihydroxyfern-9(11)-ene.

The second compound, yellow needles, was assumed to be 2-methyl-1,3,6-trihydroxyanthraquinone from its physical and spectral data, and this was confirmed by comparison with published values (6).

The third compound, reddish yellow needles, was concluded to be 1,2-dihydroxyanthraquinone (alizarin) from its physical and spectral data; this was confirmed by comparison with published values (6).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All melting points were determined on a Yanagimoto micro melting point apparatus and are recorded uncorrected. Uv spectra were recorded on a Hitachi 220 S double beam spectrophotometer, and ir spectra were obtained on a Hitachi 260-10 ir spectrometer with polystyrene calibration at 1601 cm^{-1}. Specific rotation was determined on a JASCO DIP-140 digital polarimeter. ^1H- and ^{13}C-nmr spectra were taken with a Varian XL-200 spectrometer at 200 MHz and 50.3 MHz, respectively, with TMS as an internal standard and are recorded in δ (ppm) units. Mass spectra were obtained on a JEOL JMS-D-200 mass spectrometer operating at 70 eV.

EXTRACTION, SEPARATION, AND ISOLATION.—The Chinese crude drug "Qián Cáo Gén" was purchased at a Chinese drug store, Tochimoto-tenkaido, in Osaka, Japan, and identified as *R. cordifolia* at the State Pharmaceutical Administration of China. The material (5 kg) was extracted with MeOH at room temperature for 3 days. The MeOH extract was partitioned between H_2O and $CHCl_3$, and the $CHCl_3$ fraction was partitioned between petroleum ether and MeOH-H_2O (90:10). The 90% MeOH extract was chromatographed on a silica gel column by $CHCl_3$ elution. The $CHCl_3$ eluate was rechromatographed on a Sephadex LH-20 column eluting with MeOH. The MeOH eluate was subjected to preparative tlc separation; it afforded **1** (4 mg), 2-methyl-1,3,6-trihydroxyanthraquinone (5 mg), and alizarin (5 mg).

CHARACTERIZATION OF **1**.—Colorless needles, mp 252-256° (MeOH); ir ν max (KBr) 3360, 2920, 1630, 1450, 1365, 1090, 1045, 1030 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr ($CDCl_3$) δ 15.41 (C-24), 15.52 (C-26), 16.07 (C-29), 16.51 (C-30), 16.77 (C-28), 21.71 (C-27), 21.91 (C-23), 22.91 (C-25), 24.53 (C-12),

TABLE 1. ^1H-nmr Data of Fern-9(11)-enes, **1**, **2**, and **3** (in $CDCl_3$, δ, J=Hz)

Protons	Compounds		
	3[a]	**1**	**2**
3-H		3.20, m	4.46, dd (J=4.2, J=10.9)
7-H		4.22, m	5.07, sex (J=3.5, J=9.5, J=9.5)
11-H	5.30, m	5.31, m	5.33, dd (J=2.0, J=6.3)
19-H		3.76, m	4.97, sex (J=5.5, J=10.3, J=10.3)
23-H_3	0.84, s	0.99, s	0.87, s
24-H_3	0.89, s	0.80, s	0.87, s
25-H_3	1.05, s	1.06, s	1.09, s
26-H_3	0.73, s	0.83, s	0.83, s
27-H_3	0.82, s	0.98, s	0.94, s
28-H_3	0.76, s	0.92, s	0.90, s
29-H_3	0.82, d	0.83, d J=6.2	0.82, d J=6.3
30-H_3	0.88, d	0.88, d J=6.2	0.89, d J=6.3
OAc			2.02 (3H) 2.05 (6H)

[a]See Nakanishi *et al.* (14).

27.75 (C-22), 27.97 (C-2), 30.30 (C-20), 31.90 (C-16), 32.98 (C-15), 36.30 (C-1), 36.42 (C-6), 36.88 (C-4), 39.23 (C-10), 39.38 (C-18), 40.89 (C-17), 43.90 (C-13), 47.93 (C-8), 51.95 (C-5), 57.03 (C-14), 57.27 (C-21), 71.40 (C-3), 74.52 (C-19), 75.14 (C-7), 116.98 (C-9), 121.11 (C-11) (tentatively assigned); ms *m/z* 458 (M^+), 440 (M-M_2O), 425 (M-H_2O-Me), 407 (M-H_2O-Me-H_2O), 389 (M-H_2O-Me-H_2O-H_2O), 271, 257, 217; *Anal.* calcd for $C_{30}H_{50}O_3$: 458.3760. Found (ms): 458.3735.

ACETYLATION OF **1**.—A mixture of **1** (2 mg), Ac_2O (0.1 ml), and pyridine (0.1 ml) was allowed to stand at room temperature overnight. The reaction mixture was worked up as usual to give a triacetate (**2**, 1.5 mg). Colorless needles, mp 228-233° (MeOH); $[\alpha]^{25}D$ −18.9° (c=0.09, $CHCl_3$); ^1H nmr see Table 1; ms *m/z* 584 (M^+), 582 (M-2H), 524 (M-AcOH), 464 (M-2AcOH), 449 (M-2AcOH-Me), 404 (M-3AcOH), 389, 313, 312, 295, 252, 237; *Anal.* calcd for $C_{36}H_{56}O_6$: 584.4077. Found (ms): 584.4180. *Anal.* calcd for $C_{36}H_{54}O_6$ (M-2H): 582.3917. Found (ms): 582.3942.

IDENTIFICATION OF ANTHRAQUINONES.—2-Methyl-1,3,6-trihydroxyanthraquinone and alizarin were identified by comparison with published physical and spectral data, respectively (6).

ACKNOWLEDGMENTS

The authors thank Mr. M. Morikoshi of our analytical center for his kind measurement of mass and ^{13}C-nmr spectra.

LITERATURE CITED

1. Chiang Su New Medical College (Ed.), *"Zhong-yao-da-ci-dian,"* Shanghai Scientic Publisher, Shanghai, 1977, p. 1567.
2. H. Kondo, *Yakugaku Zasshi*, **19**, 527 (1899).
3. Y. Takagi, *J. Chem. Soc. Japan*, **82**, 1561 (1961).
4. A.R. Burnett and R.H. Thomson, *J. Chem. Soc.* (C), 854 (1986).
5. V.V.S. Murti, T.R. Seshadri, and S. Sivakumaran, *Phytochemistry*, **11**, 1524 (1973).
6. H. Itokawa, K. Mihara, and K. Takeya, *Chem. Pharm. Bull.*, **31**, 2353 (1983).
7. M.I. Barisov, N.V. Kovalev, and V.G. Zaitsev, *Khim. Prir. Soedin*, **7**, 529 (1971).
8. J. Raynaud and H. Mnajed, *C.R. Acad. Sci., Ser* D, **274**, 1746 (1972).
9. K.B. Horvath, F. Hetenyi, A. Kocsis, L. Szabo, M.V. Balazs, I. Jr. Mathe, and P. Tetenyi, *Phytochemistry*, **21**, 2917 (1982).
10. P. Lucyma, *Pol. J. Pharmacol. Pharm.*, **25**, 465 (1973).
11. H. Itokawa, K. Takeya, N. Mori, T. Hamanaka, T. Sonobe, and K. Mihara, *Chem. Pharm. Bull.*, **32**, 284 (1984).
12. M. Arisawa, M. Nimura, A. Ikeda, H. Ueno, T. Hayashi, and N. Morita, *Shoyakugaku Zasshi*, **39**, 246 (1985).
13. K. Nishimoto, M. Ito, S. Natori, and T. Ohmoto, *Tetrahedron*, **24**, 735 (1968).
14. K. Nakanishi, T. Goto, S. Ito, S. Natori, and S. Nozoe, "Natural Product Chemistry," vol. 1. Kodansha, Tokyo, Academic Press, New York, 1974, p. 365, 377.
15. G.B. Marcelle, G.A. Cordell, N.R. Farnsworth, and R. Fonnegra, *Planta Med.*, **46**, 190 (1982).

Received 28 April 1986