Phytochemistry, Vol. 27, No. 12, pp. 3975–3976, 1988.
Printed in Great Britain.

0031–9422/88 $3.00 + 0.00
Pergamon Press plc.

A TRITERPENE FROM THE FRUITS OF *RUBUS CHINGII*

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(*Received* 19 *April* 1988)

Key Word Index—*Rubus chingii*; Rosaceae; diosphenol-type triterpene; fupenzic acid.

Abstract—A new diosphenol-type triterpene fupenzic acid, was isolated from the fruits of *Rubus chingii* and characterized as 2,19α-dihydroxy-3-oxo-urs-1,12-dien-28-oic acid on the basis of UV, MS, ^{1}H and ^{13}C NMR spectral analysis as well as chemical conversion.

INTRODUCTION

The fruits of *Rubus chingii* have been used as a food and tonic in traditional Chinese medicine. Tanaka *et al.* [1] have reported the isolation of a diterpene glycoside, goshonoside-F_5, from a commercial crude drug, Fu-penzi in Chinese, which was derived from the fruits of *Rubus chingii*. During the course of studies on evaluation of crude drugs from *Rubus* spp. we isolated a new diosphenol-type triterpene.

RESULTS AND DISCUSSION

A methanol extract of the roots of *Rubus chingii* was partitioned against various organic solvents and the ethyl acetate fraction was subjected to column chromatography to afford a crystalline compound **1**, along with β-sitosterol and tiliroside. Compound **1** was obtained as colourless needles, mp 189–190°. The molecular formula was determined to be $C_{30}H_{44}O_5$ by high-resolution MS. The ^{1}H NMR spectrum was characteristic of ursane-type triterpene with two olefinic protons (δ5.35 and 6.29). Furthermore, the ^{13}C NMR spectral data also indicated their similarity with 19α-hydroxyurs-12-en-28-oic acid derivatives, except for the signals ascribed to the A-ring. The UV spectrum showed an intense band at 271 nm, which shifted bathochromically to 309 nm in alkaline medium (0.1 M NaOH), whilst acetylation of **1** changed the absorption to 237 nm in the monoacetate (**2**), suggesting the presence of the diosphenol chromophore [2]. The observation of ^{13}C NMR signals at δ130.9 (C-1), 146.7 (C-2) and 203.2 (C-3) and long range carbon–proton shift correlation between C-1 and H-25 confirmed the diosphenol moiety in the A-ring. Reduction of **1** with sodium borohydride [3] afforded a triol (**3**), which had an almost identical MS spectrum with that of authentic 2α,3β,19α-trihydroxy-urs-12-en-28-oic acid but showed different chemical shifts and signal patterns at δ3.14

Table 1. ^{13}C NMR spectral data for compounds **1–3** (δ relative to TMS in CD_3OD)

C	1*	2†	3†
1	130.9 (*d*)	147.1 (*d*)	46.3 (*t*)
2	146.7 (*s*)	144.7 (*s*)	73.0 (*d*)
3	203.2 (*s*)	200.5 (*s*)	80.5 (*d*)
4	46.2 (*s*)	47.3 (*s*)	38.7 (*s*)
5	55.9 (*d*)	55.2 (*d*)	57.6 (*d*)
6	20.8 (*t*)	20.8 (*t*)	20.2 (*t*)
7	34.8 (*t*)	34.6 (*t*)	35.1 (*t*)
8	40.2 (*s*)	41.4 (*s*)	40.0 (*s*)
9	44.9 (*d*)	43.8 (*d*)	43.8 (*d*)
10	43.7 (*s*)	43.8 (*s*)	43.6 (*s*)
11	25.4 (*t*)	25.3 (*t*)	25.7 (*t*)
12	129.8 (*d*)	129.5 (*d*)	130.4 (*d*)
13	141.1 (*s*)	141.3 (*s*)	140.7 (*s*)
14	42.5 (*s*)	42.6 (*s*)	42.0 (*s*)
15	30.3 (*t*)	30.3 (*t*)	30.3 (*t*)
16	27.3 (*t*)	27.4 (*t*)	27.5 (*t*)
17	48.0 (*s*)	48.0 (*s*)	48.0 (*s*)
18	56.0 (*s*)	56.1 (*s*)	55.9 (*s*)
19	74.3 (*s*)	74.3 (*s*)	74.4 (*s*)
20	43.7 (*d*)	43.8 (*d*)	43.8 (*d*)
21	28.0 (*t*)	28.1 (*t*)	28.1 (*t*)
22	39.6 (*t*)	39.7 (*t*)	39.8 (*t*)
23	28.9 (*q*)	29.0 (*q*)	31.1 (*q*)
24	23.0 (*q*)	22.8 (*q*)	18.4 (*q*)
25	21.0 (*q*)	21.1 (*q*)	17.7 (*q*)
26	18.6 (*q*)	18.7 (*q*)	18.7 (*q*)
27	25.6 (*q*)	25.5 (*q*)	25.7 (*q*)
28	182.8 (*s*)	183.0 (*s*)	183.0 (*s*)
29	27.9 (*q*)	27.9 (*q*)	27.9 (*q*)
30	17.3 (*q*)	17.3 (*q*)	17.3 (*q*)
Me CO-		20.3 (*q*) 171.6 (*s*)	

*Measured at 100 MHz.
†Measured at 22.5 MHz.

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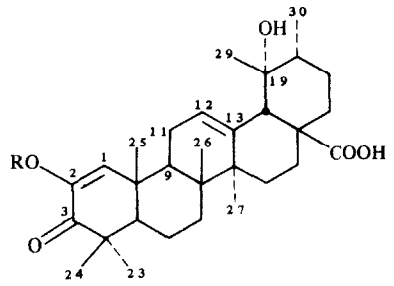

1 R = H
2 R = MeCO

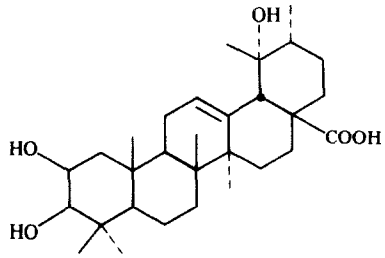

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Fig. 1. Structures of compounds 1–3.

and 4.00 (H-2 and H-3, respectively) in the ^{1}H NMR spectrum. The coupling constant between H-2 and H-3 (J = 3.9 Hz) was in agreement with the stereochemistry of the two hydroxy groups at 2β,3β [4]. This spectroscopic and chemical evidence led us to propose the structure of **1** as 2,19α-dihydroxy-3-oxo-urs-1,12-dien-28-oic acid, a new compound named fupenzic acid. Various glucosyl esters of 19α-hydroxyursane-type triterpene have been reported as common constituents of the leaves of *Rubus* spp, [5] but no diosphenol-type triterpene has been isolated hitherto.

EXPERIMENTAL

Mps: uncorr. ^{1}H NMR spectra were taken at 270 or 400 MHz, and ^{13}C spectra at 22.5 or 100 MHz. EIMS were recorded on a probe at 70 eV.

Plant material. The fruit of *Rubus chingii* was purchased from Tochimoto Tenkaido Co. Ltd. (Japan) and the botanical source was anatomically confirmed by T. N.

Extraction and isolation. The powdered, dry fruits of *R. chingii* (3 kg) was refluxed with MeOH for 3 hr (× 3). The extract (260 g) was suspended in H_2O and successively extracted with hexane, EtOAc, *n*-BuOH to give the respective extracts (27.9, 36.7 and 86.8 g). A part of the EtOAc extract (35 g) was subjected to CC on silica gel with $CHCl_3$ and $CHCl_3$–MeOH (10:1 and 2:1) to afford β-sitosterol (20 mg), tiliroside (650 mg) and a new triterpene (42 mg), respectively.

Fupenzic acid (**1**). Mp 189–190°, $[\alpha]_D^{25}$: 45.6° (MeOH, *c* 0.16), High-resol. MS, Found, *m/z* 484.3164; Calcd for M^+, $C_{30}H_{44}O_5$: 484.3187. UV λ_{max}^{MeOH} nm: 271 (log ε = 4.04). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1690, 1670 (COOH, conjugated C=O), 1640 (C=C). ^{1}H NMR (400 MHz, CD_3OD): δ0.87 (3H, *s*, H-26 × 3), 0.93 (3H, *d*, *J* = 6.6 Hz, H-30 × 3), 1.11 (3H, *s*, H-24 × 3), 1.18 (3H, *s*, H-23 × 3), 1.20 (3H, *s*, H-29 × 3), 1.22 (3H, *s*, H-25 × 3), 1.35 (3H, *s*, H-27 × 3), 2.00 (1H, *dd*, *J* = 6.8, 11.2 Hz, H-9), 2.13 (1H, *m*, H-11a), 2.23 (1H, *m*, H-11b), 2.52 (1H, *s*, H-18), 5.35 (1H, *br t*, *ca* 0.4 Hz, H-12), 6.28 (1H, *s*, H-1). Assignments were based on the ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and long-range ^{1}H–^{13}C COSY experiments. EIMS *m/z* (rel. int.): 484 (40, M^+), 438 (92), 366 (52), 201 (25), 151 (43), 146 (52), 55 (base peak).

Acetylation of **1**. Compound **1** (11 mg) was reacted with $(Ac_2)O$ (0.5 ml) in pyridine (0.5 ml) at room temp. The product was purified by prep TLC to yield a monoacetate (**2**, 8 mg): mp 168–169°. UV λ_{max}^{MeOH} nm: 237 (log ε = 3.79). ^{1}H NMR (270 MHz, CD_3OD): δ0.80, 1.03, 1.08, 1.10, 1.17, 1.27 (each 3H, *s*, Me), 0.83 (3H, *d*, *J* = 6.6 Hz, H-30 × 3), 2.07 (3H, *s*, Ac–O–), 2.43 (1H, *s*, H-18), 5.25 (1H, *br s*, H-12), 6.73 (1H, *s*, H-1). EIMS (rel. int.) *m/z*: 526 (11, M^+), 480 (82), 438 (36), 408 (56), 220 (38), 146 (100).

Reduction of **1** *with* $NaBH_4$. Compound **1** (10 mg) was reduced with $NaBH_4$ (15 mg) in MeOH (1.5 ml) for 1 hr at room temp. After addition of a few drops of HOAc, the reaction mixture was concd *in vacuo* and extracted with EtOAc. The extract was purified by prep TLC to give a crystalline compound (**3**): mp 276–278°. $[\alpha]_D^{25}$: 99.4° (MeOH, *c* 0.17). IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1690 (COOH), 1640 (C=C) ^{1}H NMR (CD_3OD): δ0.80, 1.00, 1.01, 1.19, 1.25, 1.32 (each 3H, *s*, Me), 0.93 (3H, *d*, *J* = 6.6 Hz, H-30 × 3), 2.50 (1H, *s*, H-18), 3.14 (1H, *d*, *J* = 3.9 Hz, H-3), 4.00 (1H, *br q*, H-2), 5.30 (1H, *br t*, *J* = 2.0 Hz, H-12). EIMS *m/z* (rel. int.): 488 (less than 1%, M^+), 470 (2), 406 (78), 352 (36), 201 (50), 187 (91), 146 (100).

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