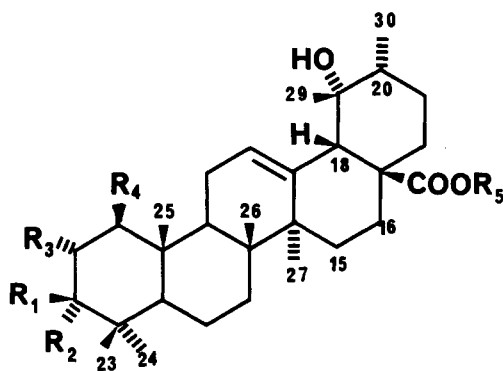


PENTACYCLIC TRITERPENES FROM THE FRUITS OF *ROSA STERILIS*LIANG GUANG-YI,¹ ALEXANDER I. GRAY, and PETER G. WATERMAN**Phytochemistry Research Laboratories, Department of Pharmacy, University of Strathclyde,
Glasgow, G1 1XW, Scotland, UK*

ABSTRACT.—The dried fruits of *Rosa sterilis* have yielded four urs-12-en-28-oic acid triterpenes, three of which were identified as pomolic acid, euscaphic acid, and kaji-ichigoside F-1. The fourth is novel and has been characterized as 1 β ,2 α ,3 α ,19 α -tetrahydroxyurs-12-en-28-oic acid (1 β -hydroxyeuscaphic acid). The value of deuteriopyridine as a solvent for ¹H-nmr studies of these triterpenes is discussed.

Rosa sterilis S.D. Shi (Rosaceae) is a newly described species recorded from Guizhou Province, China and known only from cultivated material (1). According to Shi (personal communication) a decoction of the fruits of this and the closely allied and sympatric *Rosa roxburghii* is used in Guizhou Province as a tonic. No chemical studies have been reported on *R. sterilis*, but *R. roxburghii* has been found to contain a number of urs-12-en-28-oic acid derivatives (2). In this paper we report the results of an investigation of the dried fruits of *R. sterilis* and the isolation of six compounds. In the course of this investigation high-field 2D correlation spectra were obtained, run in deuteriopyridine. The value of this solvent in nmr studies of urs-12-en-28-oic acids is discussed.

The EtOH extract of the dried fruit yielded β -sitosterol, daucosterol, and four urs-12-en-28-oic acid derivatives, three of which were identified as pomolic acid [1], euscaphic acid [2], and kaji-ichigoside F-1 [3] on the basis of physical and spectroscopic data. The high-field ¹H-nmr spectra of compounds 1–3 were run in deuteriopyridine, and chemical shift values are listed in Table 1. The ¹³C-nmr spectrum of 2 was also obtained and chemical shifts for individual carbons (see Experimental) established by ¹H-¹³C correlation spectroscopy. This confirmed the previously reported assignments for 2 (3) and resolved outstanding problems regarding the methylene carbons C-16 and C-21 and the methyl carbons C-25, C-26, and C-30.



	R ₁	R ₂	R ₃	R ₄	R ₅
1	OH	H	H	H	H
2	H	OH	OH	H	H
3	H	OH	OH	H	glucose
4	H	OH	OH	OH	H

¹Department of Phytochemistry, Guiyang College of Traditional Chinese Medicine, Guiyang, People's Republic of China.

TABLE 1. ¹H nmr Chemical Shift Data for Compounds 1-4.^a

Proton	1	2	2-AC ₂	3	4
H-1α (ax)	—	1.75 t (11.8)	ca. 1.40	1.76 t (12.0)	4.11 d (9.5)
H-1β (eq)	—	1.89 dd (12.0, 4.8)	ca. 1.50	1.91 dd (12.1, 4.1)	—
H-2α (eq)	ca. 1.90	—	—	—	—
H-2β (ax)	—	4.30 ddd (10.3, 4.1, 3.0)	5.27 ddd (9.3, 4.9, 2.7)	—	4.15 dd (9.5, 2.8)
H-3α (ax)	3.45 dd (10.4, 5.6)	—	—	—	—
H-3β (eq)	—	3.76 d (2.5)	4.93 d (2.5)	3.76 s	—
H-9	—	—	—	—	3.86 d (2.8)
H-11α (eq)	—	—	—	—	2.38 dd (11.0, 6.6)
H-11β (ax)	—	—	—	—	3.19 dt (13.1, 4.4)
H-12	5.62 t (3.5)	—	—	—	2.64 ddd (13.1, 11.0, 3.4)
H-15β (ax)	2.35 dt (13.5, 4.6)	5.58 s	5.31 t (3.3)	5.34 s	5.70 t (3.4)
H-16α (ax)	3.14 dt (13.1, 4.4)	2.33 dt (13.5, 4.8)	—	2.48 dt (13.5, 4.4)	2.36 dt (13.5, 4.5)
H-16β (eq)	2.15 dt (13.3, 4.0)	3.10 dt (13.1, 4.4)	2.49 dt (13.1, 4.4)	3.08 dt (13.2, 4.5)	3.16 dt (13.1, 4.4)
H-18	3.07 s	—	—	—	—
23-Me	1.24 s	3.04 s	2.50 s	2.92 s	3.05 s
24-Me	1.03 s	1.26 s	0.84 s	1.25 s	1.27 s ^b
25-Me	0.93 s	0.90 s	0.94 s	0.91 s	0.94 s
26-Me	1.12 s	0.98 s	1.00 s	1.03 s	1.25 s ^b
27-Me	1.74 s	1.11 s	0.69 s	1.21 s	1.22 s ^b
29-Me	1.46 s	1.64 s	1.16 s	1.60 s	1.68 s
30-Me	1.12 d (6.5)	1.42 s	1.27 s	1.37 s	1.42 s
H-1'	—	1.11 d (6.5)	0.91 d (6.7)	1.05 d (6.7)	1.11 d (6.7)
Ac	—	—	1.92/2.08	6.30 d (8.0)	—

^aAll spectra run at 360 MHz, solvent deuteriopyridine except for the diacetate of 2 which was run in CDCl₃. Coupling constants given in parentheses.^bAssignments interchangeable.

The final compound ($C_{30}H_{48}O_6$) showed its absorption bands for OH and C=O, and the 1H -nmr spectrum (Table 1) indicated the presence of seven methyls, six singlets and a doublet, suggesting an ursane derivative with one oxidized methyl. The eims showed major ions at m/z 264 [$C_{16}H_{24}O_3$] $^+$ and m/z 239 [$C_{14}H_{23}O_3$] $^+$ for retro-Diels-Alder fragmentation in ring C, typical of a Δ^{12} -pentacyclic triterpene. The former ion is characteristic of a ring D/E fragment that was also observed in **1** and **2**; the latter ion must be derived from the ring A/B fragment which, thus, also contains three oxygens.

Detailed analysis of the 1H -nmr spectrum (Table 1) revealed the characteristic features of a 19-hydroxyurs-12-en-28-oic acid. The only distinctive feature was in the occurrence of three oxymethine protons resonating at δ 3.86 d, 4.15 dd, and 4.11 d. Spin decoupling experiments revealed that these form a -CH(OH)-CH(OH)-CH(OH)- system that must be assigned to C-1-C-3. Coupling constants required two adjacent oxymethine protons to be axial and the third equatorial. These were assigned as H-3 (equatorial), H-2 (axial), and H-1 (axial) on two counts: (a) the close similarity of the H-3 and Me-23/24 resonances to those of **2** (but not **1**) and (b) the occurrence of three further deshielded protons, in the form of an ABX-system associated with the olefinic H-12. These were assigned to H-11 equatorial (δ 3.19), H-11 axial (δ 2.64), and H-9 (δ 2.38). The latter is not visible in **1-3**, and the deshielding must be associated with an equatorial hydroxyl function at C-1. On this basis the structure of the final compound must be **4**, 1 β -hydroxyeuscaphic acid.

A feature of the 1H -nmr spectra of **1-4** was the occurrence of two doublets of triplets (1H each), indicative of axial protons (Table 1), at ca. δ 2.4 and ca. δ 3.1. These have not been noted previously in spectra of urs-12-ene triterpenes as they appear only in spectra run in deuteriopyridine. They were assigned by means of a C/H correlation nmr spectrum obtained for **2**. This linked the C-15 resonance at δ_c 28.2 (3,4) with the δ 2.36 dt, which can thus be assigned as H-15 axial (β), and the C-16 resonance at δ_c 25.4 with the δ 3.10 dt, which can similarly be assigned to the H-16 axial (α).

The relationship between 1H -nmr resonances was further explored by means of COSY and long-range COSY experiments performed on **2** (Figure 1). Thus, H-16 β was located at δ 2.10 and exhibited W-bond coupling to H-18, confirming the equatorial nature of H-18 at the *cis* D/E ring junction. Similarly, H-15 β was found to exhibit W-bond coupling to Me-27. Long-range COSY studies also led to identification of individual methyls in **2** and the acetate of **2**. For the latter, run in $CDCl_3$, the order of chemical shift was Me-26 < 23 < 24 < 25 < 27 < 29, which is in agreement with published data (4,5). By contrast, for **2** run in deuteriopyridine, the order becomes Me-24 < 25 < 26 < 23 < 29 < 27. Insufficient material was available for a comparable long-range COSY study of **4**, so that assignment of resonances for Me-23, 25, and 26 remains unresolved.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were measured on a Kofler Hotstage and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Ir spectra were run as KCl discs. 1H - and ^{13}C -nmr spectra were recorded on Bruker WH-250 and WH-360 instruments (solvents and field strengths are given in the text). Hreims were recorded on an AEI MS-902 spectrometer operating at 70 eV using direct probe insertion at elevated temperatures.

PLANT MATERIAL.—The fruits of *R. sterilis* were collected by Mr. S.D. Shi from plants growing in the Guizhou Botanic Gardens, China. A voucher specimen has been retained at the Herbarium of the Botanic Gardens.

ISOLATION PROCEDURES.—Dried ground fruits (2.5 kg) from which seeds had been removed were extracted with 95% EtOH. The resulting extract was concentrated and partitioned with EtOAc. Cc of the EtOAc extract over Si gel gave, on elution with $CHCl_3$, and then $CHCl_3$ containing increasing amounts of

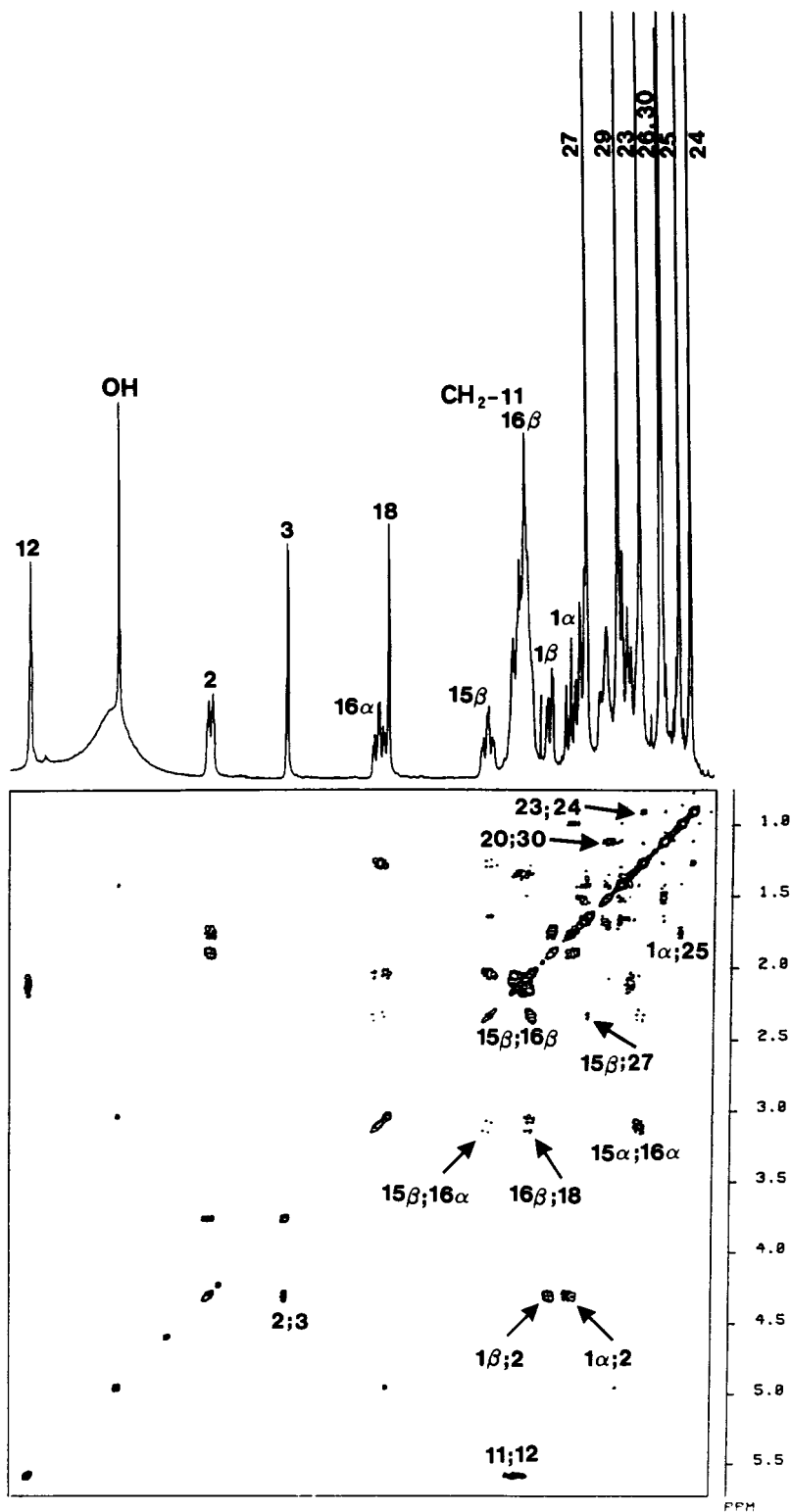


FIGURE 1. 2D COSY spectrum of euscaphic acid [2] showing 3J and 4J couplings.

MeOH: (a) with CHCl_3 , β -sitosterol (107 mg); (b) with 2% MeOH, impure **1** which was subjected to a repeat of the column procedure to give **1** (18 mg); (c) with 4% MeOH, **2** (440 mg); (d) with 7% MeOH, a mixture from which **3** (18 mg) was obtained by further cc over Si gel with CHCl_3 containing increasing amounts of MeOH; (e) with 10% MeOH, daucosterol (164 mg); (f) with 15% MeOH, **4** which was purified by further cc eluting with 10% MeOH to give 11 mg of pure **4**.

KNOWN COMPOUNDS.—Sitosterol was identified by comparison (mmp, ir, $[\alpha]_D$, ^1H nmr) with an authentic sample. Daucosterol was identified by comparison (mmp, $[\alpha]_D$, ^1H nmr of peracetate) with an authentic sample. Pomolic acid [**1**] as plates from $\text{CHCl}_3/\text{MeOH}$: mp 284° [lit. (6) $301\text{--}303^\circ$]; $[\alpha]_D + 39^\circ$ ($c = 0.3$, EtOH) [lit. (6) $+37^\circ$]; eims m/z 472.3553 $[\text{M}]^+$, calcd for $\text{C}_{30}\text{H}_{48}\text{O}_4$, 472.3552; ^1H nmr see Table 1. Euscaphic acid [**2**]: $[\alpha]_D + 20^\circ$ ($c = 0.4$, EtOH) [lit. (3) $+20^\circ$]; eims m/z 488.3502 $[\text{M}]^+$, calcd for $\text{C}_{30}\text{H}_{48}\text{O}_5$, 488.3502; ^1H nmr see Table 1; ^{13}C -nmr (90.56 MHz, deuteriopyridine) s at 37.7 (C-10), 37.8 (C-4), 39.6 (C-8), 41.8 (C-14), 47.3 (C-17), 71.8 (C-19), 138.9 (C-13), and 179.7 (C-28), d at 41.2 (C-20), 46.6 (C-9), 47.7 (C-5), 53.6 (C-18), 65.2 (C-2), 78.3 (C-3), and 127.0 (C-12), t at 17.7 (C-6), 23.1 (C-11), 25.4 (C-16), 26.0 (C-21), 28.2 (C-15), 32.5 (C-7), 37.5 (C-22), and 41.4 (C-1), q at 15.7 (C-30), 15.8 (C-25), 16.3 (C-26), 21.4 (C-24), 23.7 (C-27), 26.2 (C-29), and 28.4 (C-23). For synthesis of euscaphic acid 2,3-diacetate, compound **2** (24 mg) was dissolved in pyridine (1 ml), and Ac_2O (1 ml) was added. After normal workup and recrystallization from MeOH, plates of the diacetate of **2** (16 mg) were obtained: mp 185° [lit. (3) $189\text{--}190^\circ$]; ^1H nmr see Table 1. Kaji-ichigoside F-1 [**3**]: Needles from MeOH, mp 205° ; $[\alpha]_D + 8^\circ$ ($c = 0.6$, EtOH) [lit. (3) 7.8°]; ^1H nmr see Table 1.

1β -HYDROXYEUSCAPHIC ACID [**4**].—Amorphous solid, $[\alpha]_D + 24^\circ$ ($c = 1.5$, EtOH); ir ν max (KBr) 3480, 1688 cm^{-1} ; ^1H nmr see Table 1; eims m/z (rel. int.) 504.3451 $[\text{M}]^+$, calcd for $\text{C}_{30}\text{H}_{48}\text{O}_6$, 504.3451 (4), 486 (50), 458 (15), 264 (8), 246 (27), 239 (6), 223 (3), 222 (2), 218 (18), 146 (100).

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