NEW TRITERPENOIDS FROM RUBIA CORDIFOLIA VAR. PRATENSIS (RUBIACEAE)

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From the roots of Rubia cordifolia var. pratensis, three new triterpenoids named rubiprasins A, B and C have been isolated and their structures were determined as 3β-acetoxyoleanane-13β,15α-diol-12-one, 3β-acetoxyoleanane-13β-ol-12-one and 3β-acetoxyl-19α-hydroxyolean-12-en-28-oic acid respectively by various chemical and spectroscopic methods including ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and X-ray diffraction.

KEYWORDS rubiprasin A; rubiprasin B; rubiprasin C; Rubia cordifolia var. pratensis; Rubiaceae; oleanane-type; triterpenoid; ^{1}H-^{1}H COSY; ^{1}H-^{13}C COSY; X-ray diffraction

The methanol extract of the roots of R. cordifolia var. pratensis[1] collected in China was partitioned between water and chloroform, and then between water and n-butanol. The chloroform-soluble fraction was applied to repeated silica gel column chromatography to give three new oleanane-type triterpenoids (rubiprasins A, B and C) shown in Fig. 1.

Rubiprasin A: colorless needles (from $CHCl_3$), mp > 300°C, $[\alpha]_D$ +12.8° in EtOH showed IR absorption bands at 3470, 3430, 2920, 2870, 1720, 1695, 1478, 1465, 1365, 1270 and 1025 cm^{-1} in KBr. The MS showed fragment ion peaks at m/z 516 $[M]^+$, 498 $[M-H_2O]^+$, 235, 207 (base peak) and 189. The ^{1}H-, ^{13}C-NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY spectra of rubiprasin A in pyridine-d_5 exhibited the presence of the partial structures 1, 2, 3, 4, 5, and 6 shown in Fig. 2. In addition to eight tertiary methyls at δ 0.88, 0.91, 0.92, 0.94, 1.01, 1.40, 1.61 and 1.85, one acetyl group [δ 2.05 and δ 21.12 (q), 170.54 (s)], one ketonic carbonyl carbon [δ 210.40 (s)], one acetoxylated and two hydroxylated carbons [δ 80.56 (d), 85.83 (s) and 66.70 (d)] and six quarternary carbons at δ 31.79, 35.06, 37.85, 37.98, 44.88 and 51.09. From these results, it was concluded this compound was a 3β-acetoxyoleanane derivative, which had the ketonic carbonyl group in the C-12 position and one of two hydroxyl groups was attached to C-13, because it was found that the carbon signal of 28-CH_3 was shifted downfield about 4 ppm by the syn-diaxial OH - CH_3 interaction[2] in comparison with β-amyrin[3], and another hydroxyl group was found to be linked to the C-15 as an α-orientation or the C-16 as a β-orientation. Also, rubiprasin A was acetylated with Ac_2O - pyridine in the usual way to give a mono-acetate, colorless needles from $CHCl_3$, mp 270 - 271°C and MS m/z; 558 $[M^+]$.

In order to confirm the structure of rubiprasin A indicated by the spectroscopic methods, its X-ray

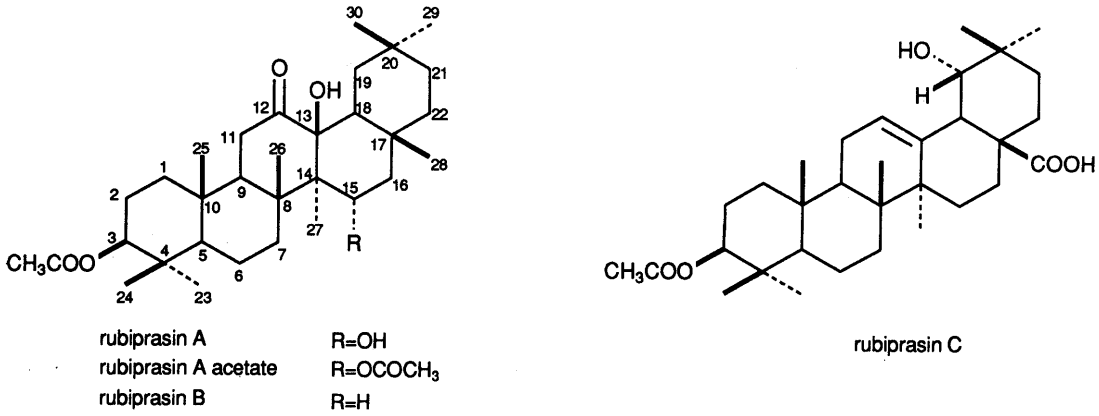

Fig. 1. Structures of Rubiprasins A, A-acetate, B and C

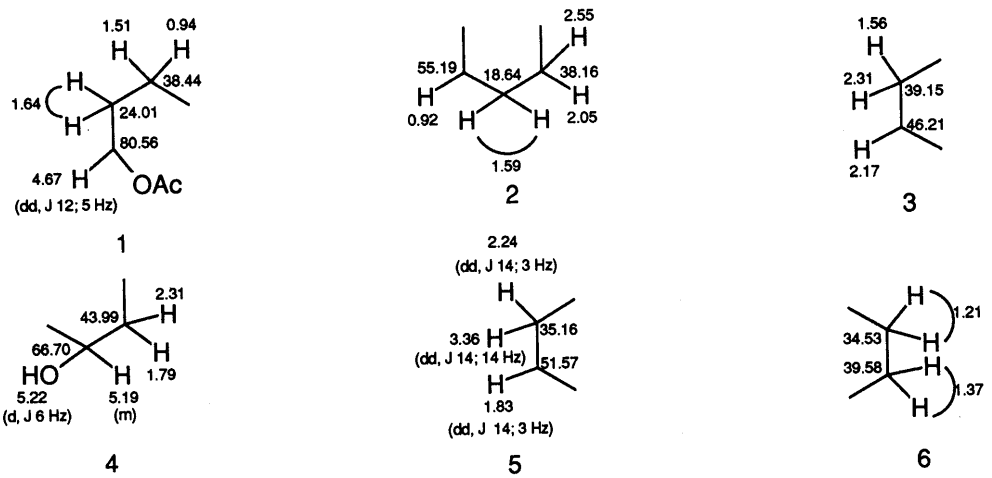

Fig. 2. Partial Structures 1, 2, 3, 4, 5 and 6 of Rubiprasin A

Crystal System: orthorhombic

Space Group: $P2_1\ 2_1\ 2_1$

z: 4

a = 11.079 (6) Å

b = 32.743 (17) Å

c = 8.245 (5) Å

v = 2991 $Å^3$

R = 0.054

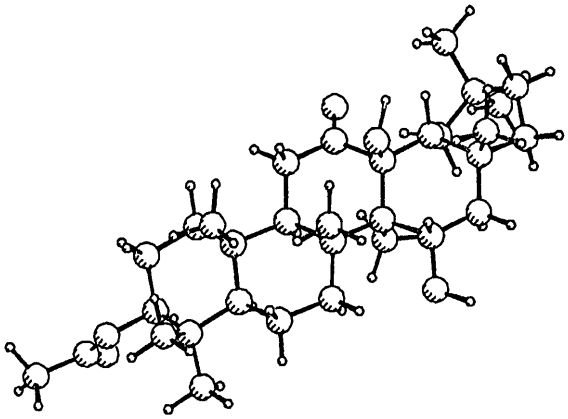

Fig. 3. Perspective View of Rubiprasin A

Table I. ^{13}C-NMR Spectral Data for Rubiprasins A, B and C (100.6 MHz, Py-d_5)

Carbon No.	A	B	C	Carbon No.	A	B	C
1	38.44 (t)	38.25 (t)	38.06 (t)	17	35.06 (s)	34.24 (s)	46.09 (s)
2	24.01 (t)	23.98 (t)	24.10 (t)[b]	18	51.57 (d)	51.32 (d)	44.81 (d)
3	80.56 (d)	80.66 (d)	80.85 (d)	19	35.16 (t)	35.47 (t)	81.30 (d)
4	37.98 (s)	38.15 (s)[a]	37.91 (s)	20	31.79 (s)	30.01 (s)	35.74 (s)
5	55.19 (d)	55.58 (d)	55.68 (d)	21	34.53 (t)	34.67 (t)	29.15 (t)[d]
6	18.64 (t)	18.30 (t)	18.65 (t)	22	39.58 (t)	39.73 (t)	33.18 (t)[c]
7	38.16 (t)	31.84 (t)	33.66 (t)[c]	23	27.96 (q)	28.06 (q)	28.16 (q)
8	44.88 (s)	42.79 (s)	39.99 (s)	24	16.71 (q)	16.68 (q)	16.89 (q)
9	46.21 (d)	46.45 (d)	48.16 (d)	25	15.94 (q)	15.89 (q)	15.33 (q)
10	37.85 (s)	37.47 (s)[a]	37.35 (s)	26	20.41 (q)	20.09 (q)	17.45 (q)
11	39.15 (t)	39.21 (t)	23.88 (t)[b]	27	14.38 (q)	18.69 (q)	24.87 (q)
12	210.40 (s)	210.28 (s)	123.24 (d)	28	32.00 (q)[e]	31.45 (q)[f]	180.86 (s)
13	85.83 (s)	83.09 (s)	144.92 (s)	29	32.27 (q)[e]	32.10 (q)[f]	28.83 (q)
14	51.09 (s)	45.39 (s)	42.16 (s)	30	24.84 (q)	25.85 (q)	24.87 (q)
15	66.70 (d)	23.08 (t)	28.39 (t)[d]	Ac-Me	21.12 (q)	21.11 (q)	21.12 (q)
16	43.99 (t)	34.03 (t)	29.24 (t)[d]	Ac-CO	170.54 (s)	170.53 (s)	170.59 (s)

a-f) Assignments may be reversed.

diffraction analysis was undertaken and it was confirmed that the structure was 3β-acetoxyoleanane-13β,15α-diol-12-one. The perspective view of rubiprasin A is shown in Fig. 3. The proton and carbon signals shown in Fig. 2 and Table I were assigned based on the ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY spectral data, and also by comparison of our spectral data with those of β-amyrin.[3]

Rubiprasin B: colorless needles (from $CHCl_3$), mp 277 - 280°C, showed fragment ion peaks at m/z 500 [M^+], 333, 249, 220, 208 (base peak) and 189 in the MS. The proton and carbon signals of C-15 hydroxymethine which appeared at δ 5.22 (OH), 5.19 (CH) and δ 66.70 (d) in the NMR of rubiprasin A was not found in the NMR of rubiprasin B, while other signals were similar to those of rubiprasin A. Also, the 27-CH_3 carbon signal (δ 14.38) in rubiprasin A was shifted downfield to δ 18.67 in rubiprasin B. It was suggested that there was no hydroxyl group at C-15 in rubiprasin B, because the γ-gauche substituent effect between 27-CH_3 and 15α-OH in rubiprasin A disappeared in rubiprasin B. Therefore, rubiprasin B was confirmed to be 3β-acetoxyoleanane-13β-ol-12-one. The carbon signals shown in Table I were assigned by comparing with rubiprasin A.

Rubiprasin C: colorless needles (from $CHCl_3$), mp 171-173°C, showed seven tertiary methyl groups at δ 0.70, 0.86, 0.86, 0.92, 0.95, 0.97 and 1.24[4], one acetyl group [δ 2.04, and δ 21.12 (q), 170.59 (s)], one carboxyl group (δ 180.86), two secondary hydroxymethine carbon signals [δ 80.85 (d) and 81.30 (d)] and one double bond [δ 5.42 (1H, t, J=3 Hz)[4] and δ 123.24 (d), 144.92 (s)]. So this compound was assumed to be a 3β-acetoxyoleanolic acid derivative. The fragment ion peaks at m/z 515 $[M+1]^+$, 263 (a characteristic retro-Diels-Alder fragment), 246 $[263-H_2O]^+$ and 201 $[263-COOH]^+$ (base peak) in the MS suggested that the D or E ring was substituted by one hydroxyl group. It was proved from the ^{1}H-^{1}H COSY spectrum that neighbors of the hydroxymethine (δ 81.30) must be linked to a quarternary carbon and methine [C-C(OH)H-CH]. Furthermore, it appeared that the hydroxyl group was bonded to C-19 as an α-orientation, because the coupling signal with the neighboring proton was a broad singlet due to the vicinal protons' angle of about 90°, and the C-12 and C-13 chemical shifts were scarcely changed in comparison with oleanolic acid,[5] whereas, if the hydroxyl group at C-19 was present as a β-orientation, their chemical shifts would vary more greatly.[6] Consequently, rubiprasin C was confirmed to be 3β-acetoxy-19α-hydroxyolean-12-en-28-oic acid. The assignment of each carbon signal is shown in Table I.

Several ursolic acid- and fernane-type triterpenes from Rubia cordifolia,[7,8] and oleanane analogs bearing a ketonic carbonyl group at C-12[9] or hydroxyl group at C-13[10] have been reported independently. However, oleanane-type triterpenes having oxygen-functions at both positions of C-12 and C-13, rubiprasins A and B, from R. cordifolia var. pratensis were isolated for the first time.

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(Received May 1, 1989)