# NEW ARBORANE TYPE TRITERPENOIDS FROM RUBIA CORDIFOLIA VAR. PRATENSIS AND R. ONCOTRICHA

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The structures of six new arborane type triterpenoids, rubiarbonols A, B, C, D, E, and F, from Rubia plants were determined as 3β,7β,19α,28-tetrahydroxyarbor-9(11)-ene, 3β,7β,19α-trihydroxyarbor-9(11)-ene, 2α-acetoxy-3β,7β,19α-trihydroxyarbor-9(11)-ene, 3β-acetoxy-2α,7β,19α-trihydroxyarbor-9(11)-ene, 2α,3β,7β,19α-tetrahydroxyarbor-9(11)-ene, and 2α,3β,7β,19α,28-pentahydroxyarbor-9(11)-ene respectively by various spectroscopic methods.

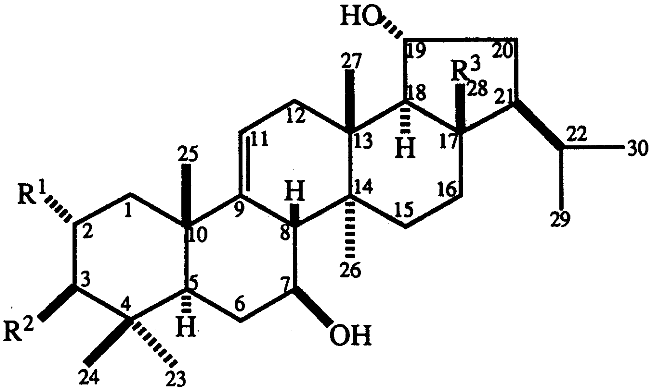
**KEYWORDS** rubiarbonol; Rubia cordifolia var. pratensis; Rubia oncotricha; Rubiaceae; arborane; triterpenoid; $^1$H-$^{13}$C COSY; COLOC

We have reported the isolation and characterization of three new oleanane type triterpenoids, rubiprasins A, B, and C which were obtained from the roots of Rubia cordifolia L. var. pratensis Maxim.[1] Here we describe the further isolation and structural elucidation of six new arborane type triterpenoids which have been isolated for the first time from the genus Rubia.

The roots of R. cordifolia var. pratensis and R. oncotricha Hand-Mazz. were collected in China. Methanol extracts were partitioned between water and chloroform, then the respective chloroform-soluble fractions were applied to repeated Sephadex LH-20 and silica gel column chromatography to give rubiarbonol A from R. cordifolia var. pratensis and rubiarbonols B, C, D, E, and F from R. oncotricha (Fig. 1).

Rubiarbonol A, colorless plates (from MeOH),[2] had partial structures 1, 2, 3, 4, 5, and 6 in the $^1$H-$^1$H and $^1$H-$^{13}$C COSY spectra (Fig. 2). There were also seven methyls, one hydroxymethyl, and six quarternary carbons in the $^1$H and $^{13}$C-NMR spectra (Tables I and II). From this it was evident that rubiarbonol A was an arborane or a fernane type triterpenoid. Also, correlation spectroscopy via $^1$H-$^{13}$C long-range coupling (COLOC) experiments at 500 MHz (in pyridine-d$_5$) gave the complete carbon signal assignments (Table II). Arborane and fernane triterpenoids have the same configurations at the 5- and 10-positions, but they have enantiomeric ones at the 8-, 13-, 14-, 17-, 18- and 21-positions.[3] So the relationship between C$_{10}$-CH$_3$ and C$_8$-H is syn-1,3-diaxial in arborane, but anti-1,3-diaxial in fernane. To determine the skeleton of rubiarbonol A, an 1D-NOE experiment was performed. As Fig. 3 shows, it must be an arborane type triterpenoid. The configurations of three hydroxyl groups linked to the 3-, 7- and 19-positions were β, β and α from the multiplicities and coupling constants of their protons (Table I). Therefore, rubiarbonol A was determined as shown in Fig. 1.

Rubiarbonol B,[4] colorless plates (from MeOH), was found to be a triterpenoid having eight methyls by the $^1$H- and $^{13}$C-NMR spectra, and it has the same partial structures as rubiarbonol A except for the partial



| Rubiarbonol | R1 | R2 | R3 |
|---|---|---|---|
| A | H | OH | CH$_2$OH |
| B | H | OH | CH$_3$ |
| C | OAc | OH | CH$_3$ |
| D | OH | OAc | CH$_3$ |
| E | OH | OH | CH$_3$ |
| F | OH | OH | CH$_2$OH |

Fig. 1. Structures of Rubiarbonols A - F

Table I. $^{1}$H-NMR Spectra Data of Rubiarbonols A – F (400 MHz, Py-$d_5$, Coupling Constants in Hz)

| | Rubiarbonol A | Rubiarbonol B | Rubiarbonol C | Rubiarbonol D* | Rubiarbonol E | Rubiarbonol F |
|---|---|---|---|---|---|---|
| H-2 | — | — | 5.54(td,10,4) | 4.29(td,10,4) | 4.21(td,11,4) | 4.21(td,11,4) |
| H-3 | 3.50(dd,10,6) | 3.49(dd,10,6) | 3.56(dd,10,5)[a] | 5.08(d,10) | 3.43(d,10) | 3.41(d,9) |
| H-6** | 2.31(ddd,13,5,2) | 2.31(ddd,13,5,2) | — | 2.20(ddd,13,5,2) | 2.30(ddd,13,5,2) | 2.29(ddd,13,5,3) |
| H-7 | 4.10(td,10,5) | 4.06(td,10,5) | 4.04(td,10,5) | 4.02(td,11,5) | 4.07(td,11,5) | 4.09(td,10,5) |
| H-11 | 5.54(brd,6) | 5.48(brd,6) | 6.50(brd,6) | 5.58(brd,6) | 5.63(brd,5) | 5.67(brd,6) |
| H-15** | 2.85(dt,15,4) | 2.82(dt,15,3) | 2.81(dt,15,3) | 2.78(dt,16,3) | 2.81(dt,15,3) | 2.82(dt,16,3) |
| H-18 | 2.37(d,10) | 2.04(d,10) | — | — | — | 2.34(d,10) |
| H-19 | 5.08(td,10,3) | 4.51(td,10,3) | 4.50(td,9,3) | 4.48(td,10,3) | 4.49(td,10,3) | 5.05(td,10,3) |
| $CH_3$-23 | 1.26(s) | 1.25(s) | 1.26(s)[b] | 1.05(s)[d] | 1.26(s)[e] | 1.28(s) |
| $CH_3$-24 | 1.12(s) | 1.11(s) | 1.14(s)[c] | 1.01(s)[d] | 1.15(s)[f] | 1.15(s) |
| $CH_3$-25 | 1.20(s) | 1.21(s) | 1.32(s)[b] | 1.23(s)[d] | 1.31(s)[e] | 1.28(s) |
| $CH_3$-26 | 1.37(s) | 1.28(s) | 1.27(s)[b] | 1.27(s)[d] | 1.28(s)[e] | 1.34(s) |
| $CH_3$-27 | 1.46(s) | 1.16(s) | 1.16(s)[c] | 1.14(s) | 1.16(s)[f] | 1.44(s) |
| $CH_3$-28 | 4.24;4.12(d,11) | 0.88(s) | 0.90(s) | 0.89(s) | 0.90(s) | 4.22;4.11(d,11) |
| $CH_3$-29 | 0.98(d,6) | 0.89(d,6) | 0.91(d,6) | 0.90(d,6) | 0.91(d,6) | 0.98(d,6) |
| $CH_3$-30 | 1.11(d,6) | 0.84(d,6) | 0.86(d,6) | 0.86(d,6) | 0.86(d,6) | 1.11(d,6) |
| $CH_3$-CO | — | — | 2.02(s) | 2.11(s) | — | — |

a) It became double (J=10Hz) when $D_2O$ was added. b–f) Assignments may be reversed.
* The coupling constants were obtained from the spectrum made in $CD_3OD$.
** These protons are equatorial.

Table II. $^{13}$C-NMR Spectra Data of Rubiarbonols A – F (100 MHz, Py-$d_5$)

| C-No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 36.98 | 28.74 | 77.99 | 39.42 | 48.99 | 33.86 | 72.13 | 49.43 | 147.69 | 39.79 | 117.29 | 37.64 | 38.37 | 40.23 | 33.02 | 33.32 |
| B | 37.03 | 28.81 | 78.04 | 39.49 | 49.07 | 33.94 | 72.20 | 49.48 | 147.64 | 39.88 | 117.09 | 37.34 | 38.33 | 39.93 | 32.08 | 37.17 |
| C | 42.53 | 74.05 | 79.47 | 40.22[a] | 48.89 | 33.77 | 71.94 | 49.14 | 146.64 | 40.98[a] | 117.46 | 37.16 | 38.34 | 39.93 | 32.06 | 37.16 |
| D | 46.57 | 66.92 | 84.80 | 39.35 | 48.72 | 33.62 | 71.84 | 49.15 | 146.71 | 40.77 | 117.55 | 37.29 | 38.32 | 39.94 | 32.07 | 37.15 |
| E | 46.02 | 69.20 | 83.57 | 39.71 | 49.12 | 33.91 | 72.07 | 49.24 | 147.24 | 41.03 | 117.26 | 37.30 | 38.35 | 39.95 | 32.10 | 37.18 |
| F | 46.04 | 69.20 | 83.58 | 39.71 | 49.09 | 33.91 | 72.06 | 49.28 | 147.38 | 41.03 | 117.52 | 37.68 | 38.46 | 40.33 | 33.11 | 33.38 |
| | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | Ac-Me | Ac-CO |
| A | 48.99 | 59.99 | 70.67 | 43.41 | 58.07 | 30.71 | 28.67 | 16.36 | 22.02 | 17.23 | 16.74 | 62.89 | 23.38 | 23.58 | — | — |
| B | 43.82 | 59.26 | 70.38 | 41.91 | 57.83 | 30.72 | 28.74 | 16.42 | 22.05 | 17.12 | 16.99 | 15.95 | 22.20 | 23.20 | — | — |
| C | 43.82 | 59.21 | 70.33 | 41.91 | 57.84 | 30.70 | 29.02 | 17.37 | 22.63 | 17.10 | 16.96 | 15.94 | 22.20 | 23.19 | 21.34 | 170.84 |
| D | 43.81 | 59.23 | 70.38 | 41.90 | 57.83 | 30.71 | 28.73 | 17.99 | 22.97 | 17.09 | 16.98 | 15.94 | 22.21 | 23.19 | 21.21 | 171.12 |
| E | 43.83 | 59.26 | 70.39 | 41.90 | 57.84 | 30.71 | 29.25 | 17.53 | 23.13 | 17.14 | 16.99 | 15.94 | 22.21 | 23.19 | — | — |
| F | 49.09 | 60.09 | 70.72 | 43.49 | 58.17 | 30.78 | 29.27 | 17.54 | 23.17 | 17.31 | 16.60 | 62.95 | 23.45 | 23.63 | — | — |

a) Assignments may be exchangeable.

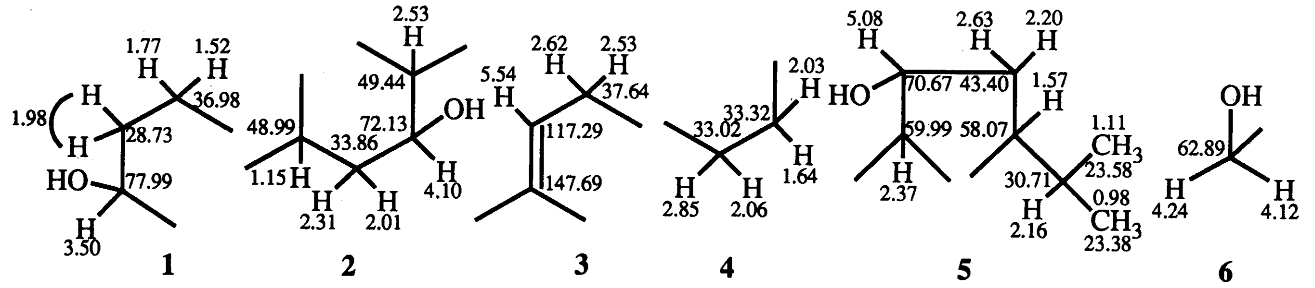


Fig. 2. Partial structures 1, 2, 3, 4, 5 and 6 of Rubiarbonol A (400 MHz, Py-$d_5$)

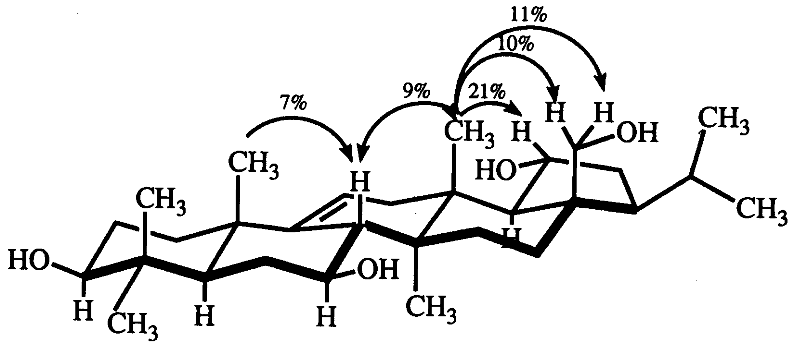


Fig. 3. NOE Enhancements of Rubiarbonol A (500MHz, Py-$d_5$)

structure 6 from the $^1H$-$^1H$ and $^1H$-$^{13}C$ data. Apparently it is an arborane type triterpenoid in analogy with rubiarbonol A. The complete assignments have been made by the analysis of the COLOC spectrum at 500 MHz (in pyridine-$d_5$). Also, a 5% enhanced NOE effect appeared between the methyl group at the C-10 and the proton at the C-8 in 1D-NOE experiment. The hydroxyl groups attached to the 3-, 7- and 19-positions evidently were $\beta$, $\beta$ and $\alpha$ orientations similar to rubiarbonol A. Consequently, rubiarbonol B was cofirmed as shown in Fig. 1.

Rubiarbonol C$^{5)}$ was isolated as colorless needles and D$^{6)}$ as colorless powder. Their $^{13}C$ chemical shifts were like those of rubiarbonol B, except for the appearance of an acetyl group in each one and the carbon signals of ring A, namely the C-2 and C-3 signals were shifted 45.24 and 1.43 ppm downfield in rubiarbonol C, and 38.11 and 6.76 ppm downfield in rubiarbonol D, compared with rubiarbonol B (Table II). So rubiarbonols C and D have an additional hydroxyl group at C-2, which has been acetylated in the former, and the C-3 hydroxyl group has been acetylated in the latter, as is also apparent from their $^1H$-NMR spectra (Table I). Thus, rubiarbonols C and D were respectively established as shown in Fig. 1. The orientations of the hydroxyl groups were also confirmed from the proton coupling constants combining with the hydroxy-carbons. The complete assignments of rubiarbonols C and D were determined on the basis of the spectral data of rubiarbonol B.

Rubiarbonols E$^{7)}$ and F$^{8)}$ were obtained as trace constituents of colorless powder. Their structures were determined in the same way as shown in Fig. 1. The structure of rubiarbonol F was also supported by the $^1H$-$^1H$ COSY spectrum.

The isolations of rubiarbonols A - F from *R. cordifolia* var. *pratensis* and *R. oncotricha* are particularly interesting in view of the fact that arborane type triterpenoids have rarely been isolated from natural sources,$^{3,9)}$ and were not yet discovered before in *Rubia* plants.

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4) Rubiarbonol B, HRMS: Calc. 440.3651, Found 440.3627, $C_{30}H_{48}O_2$ ($M^+$-$H_2O$), mp 272 - 273°C, $[\alpha]_D$ +30.3° (c 0.17, MeOH), IR$\nu_{max}$cm$^{-1}$(KBr): 3338 (OH), 1640 (C=C).
5) Rubiarbonol C, HRMS: Calc. 498.3706, Found 498.3699, $C_{32}H_{50}O_4$ ($M^+$-$H_2O$), mp 211 - 213°C, $[\alpha]_D$ -4.3° (c 0.70, MeOH), IR$\nu_{max}$cm$^{-1}$(KBr): 3436 (OH), 1719 (C=O), 1639 (C=C).
6) Rubiarbonol D, HRMS: Calc. 498.3706, Found 498.3659, $C_{32}H_{50}O_4$ ($M^+$-$H_2O$), mp 218 - 220°C, $[\alpha]_D$ +6.1° (c 0.33, MeOH), IR$\nu_{max}$cm$^{-1}$(KBr): 3423 (OH), 1740 (C=O), 1720 (chelate C=O), 1638 (C=C).
7) Rubiarbonol E, HRMS: Calc. 456.3600, Found 456.3506, $C_{30}H_{48}O_3$ ($M^+$-$H_2O$), dec. 290°C, $[\alpha]_D$ +15.4° (c 0.13, MeOH), IR$\nu_{max}$cm$^{-1}$(KBr): 3423 (OH), 1630 (C=C).
8) Rubiarbonol F, HRMS: Calc. 472.3550, Found 472.3551, $C_{30}H_{48}O_4$ ($M^+$-$H_2O$), dec. 280°C, $[\alpha]_D$ +33.3° (c 0.09, MeOH), IR$\nu_{max}$cm$^{-1}$(KBr): 3432 (OH), 1631 (C=C).
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