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# TWO PENTACYCLIC TRITERPENES FROM *RUBIA CORDIFOLIA**

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**Key Word Index**—*Rubia cordifolia*; Rubiaceae; triterpenes; rubicoumaric acid; rubifolic acid; $^{13}C$ NMR.

**Abstract**—Rubicoumaric acid and rubifolic acid isolated from *Rubia cordifolia* have been shown to be 30-hydroxy-3$\beta$-*p*-hydroxycoumaryloxy-urs-12-ene-28-oic acid and 3$\beta$,30-dihydroxy-urs-12-ene-28-oic acid(30-hydroxyursolic acid) respectively on the basis of $^1H$ NMR, $^{13}C$ NMR and mass spectral and chemical evidence.

## INTRODUCTION

*Rubia cordifolia* L. (Rubiaceae) is a small plant [2] growing throughout India in hilly districts; roots are reported to be used as a tonic and the stems as an antidote for cobra bite and scorpion sting. *R. cordifolia* elaborates anthraquinone derivatives [3]. However, no report is available on its triterpene constituents.

The plant materials for the present study were collected from the Darjeeling district, West Bengal (voucher: Herbarium of the Lloyd's Botanic Garden, Darjeeling). The isolation of two new triterpenes from this plant and the spectral and chemical evidence leading to the elucidation of their structures and stereochemistry are discussed in this paper.

## RESULTS AND DISCUSSION

The $CHCl_3$ extract of the defatted whole plant on chromatography over silica gel yielded from the MeOH–$CHCl_3$ (5:95) eluate fractions rubicoumaric acid (**1**) (0.02% yield) and a small amount of rubifolic acid (**5**), mp 286–287°, $C_{30}H_{48}O_4$ ($M^+$ 472). Rubicoumaric acid showed a positive Liebermann–Burchardt colour test for triterpenes. Through repeated chromatography over silica gel, it was obtained in a pure and homogeneous but amorphous state, mp 215°, $[\alpha]_D$ + 24.5° (EtOH), $M^+$ at *m/z* 618 ($C_{39}H_{54}O_6$); $\nu_{max}$ (KBr) 3400 (OH), 1710 (sh, conjugated ester C=O), 1690 (COOH), 1625 (phenyl conjugated C=C), 1605 and 1595 (aromatic), 1170 (ester C—O), 997 (*trans* CH=CH) and 833 $cm^{-1}$ (1,4-disubstituted benzene). Acetylation ($Ac_2O$–pyridine) formed rubicoumaric acid diacetate (**2**), crystallizing from $CHCl_3$–petrol as colourless needles, mp 205°, $[\alpha]_D$ + 12°($CHCl_3$); $C_{43}H_{58}O_8$ ($M^+$ 702); 1765 (phenolic acetate), 1725 (alcoholic acetate), 1710 (conjugated ester C=O), 1690 $cm^{-1}$ (COOH).

The 80 MHz $^1H$ NMR spectrum of **2** in $CDCl_3$ confirmed the presence of five tertiary methyls on saturated carbons ($\delta$ 0.78, 0.94 and 1.07, 3 H, *s* each; 0.86, 6 H, *s*), one secondary methyl on a saturated carbon (0.92, 3 H, *d*, *J* = 7.5 Hz), one phenolic acetate methyl (2.24 3 H, *s*), one primary acetate group (1.98, 3 H, *s*, $CH_2OCOCH_3$; 3.85–4.35, 2H, *m* $CHCH_2OCOMe$), one deshielded methine proton on C-3 bearing a OCOR group (4.37, 1 H, *dd*, *J* ≈ 12, 8 Hz), one olefinic proton having vicinal and allylic protons (5.24, 1 H, *br s*, $W_{\frac{1}{2}}$ = 8 Hz), a *trans*-disubstituted double bond (6.38, 1 H, *d*, $H_A$; 7.66, 1 H, *d*, $H_B$, $J_{A,B}$ = 16 Hz, *trans*-$ArCH_B{=}CH_ACOOH$) and a 1,4-disubstituted phenyl group (7.13, 2 H, *d*, H-3′ and H-5′, $J_{ortho}$ = 8.3 Hz; 7.55, 2 H, *d*, H-2′ and H-6′, $J_{ortho}$ = 8.4 Hz. The mass spectra and $^{13}C$ NMR spectra (Tables 1 and 2) showed that the $CH_2OAc$ was attached to the chiral C-20.

Table 1. Mass spectral data of rubicoumaric acid (**1**), methyl rubifolate (**7**) and their derivatives

| Compound | a | b | c | d* | e | f | b–ROH | b–$CO_2R'$ | b–ROH –$CO_2R'$ | e–ROH |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 353 (2)† | 264 (100) | 251 (1.9) | — | 205 (2.9) | 133 (9.1) | 246 (6.3) | 219 (3.7) | 201 (44.5) | 187 (8.9) |
| **2** | 395 (8.3) | 306 (23) | 293 (11) | 396 (8.3) | 247 (23) | 133 (41.4) | 246 (96) | 261 (3) | 201 (95) | 187 (32) |
| **4** | 249 (12.5) | 320 (85) | 307 (11.2) | 250 (2.4) | 247 (9.3) | 133 (11.6) | 260 (18.4) | 261 (8.6) | 201 (100) | 187 (20.5) |
| **6** | 249 (5.9) | 306 (14.6) | 293 (1.9) | 250 (1.6) | 247 (2.6) | 133 (10.7) | 246 (11.6) | 261 (1.7) | 201 (15.2) | 187 (11.2) |
| **7** | 207 (38.9) | 278 (75.6) | 265 (8.2) | 208 (9.5) | 247 (27.5) | 133 (14.6) | 260 (5.8) | 219 (12.3) | 201 (100) | 187 (19.1) |

*ion **d**: Me in place of $CH_2$ in ion **a**.
†*m/z* (rel. int.).

*Part XVIII in the series "Terpenoid and Related Compounds". For Part XVII see ref. [1].

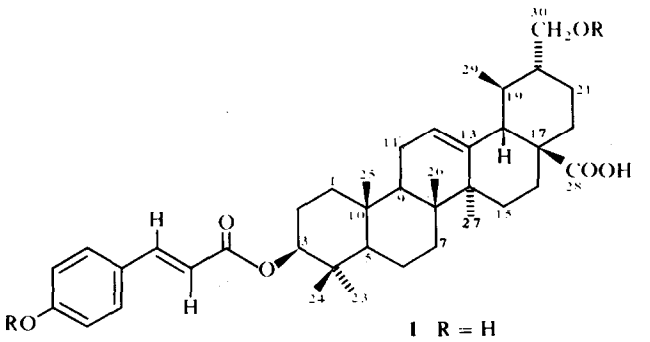
Table 2. $^{13}C$ NMR chemical shifts of compound **2**, **4**, some related urs-12-enes (**8** and **9**) and an olean-12-ene (**10**) in $CDCl_3$*

| | Carbon | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| **2** | 38.0 | 23.0 | 80.6 | 37.4 | 55.0 | 17.9 | 32.6 | 39.3 | 47.3 | 36.6 |
| **8** | 38.1 | 23.8 | 80.8 | 37.5 | 55.1 | 18.0 | 32.7 | 39.3 | 47.3 | 36.9 |
| **4** | 38.0 | 23.1 | 80.4 | 37.3 | 55.0 | 17.8 | 32.6 | 39.2 | 47.1 | 36.5 |
| **9**† | 38.3 | 23.6 | 80.7 | 37.6 | 55.3 | 18.1 | 32.8 | 39.5 | 47.4 | 36.8 |
| **10**† | 38.1 | 23.6 | 80.7 | 37.5 | 55.2 | 18.2 | 32.6 | 39.3 | 47.5 | 36.9 |
| | Carbon | | | | | | | | | |
| | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| **2** | 23.4 | 126.2 | 137.1 | 41.7 | 27.8 | 23.8 | 47.3 | 52.0 | 33.7 | 43.1 |
| **8** | 23.4 | 125.5 | 137.8 | 41.6 | 27.9 | 23.9 | 47.8 | 52.3 | 38.8 | 38.7 |
| **4** | 23.2 | 125.8 | 137.3 | 41.6 | 27.2 | 23.3 | 47.4 | 52.3 | 33.5 | 42.9 |
| **9**† | 23.2 | 125.4 | 138.0 | 41.9 | 28.1 | 24.2 | 48.0 | 52.8 | 38.9 | 38.9 |
| **10**† | 23.0 | 122.1 | 143.6 | 41.6 | 27.7 | 23.6 | 46.6 | 41.3 | 45.8 | 30.6 |
| | Carbon | | | | | | | | | |
| | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| **2** | 24.7 | 35.8 | 27.8 | 16.9 | 15.3 | 16.4 | 23.4 | 183.1 | 16.4 | 67.4 |
| **8** | 30.4 | 36.5 | 27.9 | 16.9 | 15.5 | 16.5 | 23.9 | 184.0 | 16.8 | 21.0 |
| **4** | 24.6 | 35.7 | 27.7 | 16.5 | 15.1 | 16.4 | 23.2 | 177.1 | 16.4 | 67.2 |
| **9**† | 30.7 | 36.6 | 28.1 | 16.9 | 15.5 | 16.9 | 23.6 | 177.6 | 17.1 | 21.2 |
| **10**† | 33.8 | 32.3 | 28.0 | 16.8 | 15.3 | 16.8 | 25.8 | 177.8 | 33.1 | 23.6 |
| | Carbon | | | | | | | | | |
| | 3-CHOCOC$H_3$ | | 3-CHOC$O$Me | | 28-$CO_2$C$H_3$ | | 30-$CH_2$OCOC$H_3$ | | 30-$CH_2$-OC$O$Me | |
| **2** | — | | — | | — | | 20.9 | | 170.7 | |
| **8** | 21.1 | | 170.7 | | — | | — | | — | |
| **4** | 20.8 | | 170.5 | | 51.0 | | 20.5 | | 170.3 | |
| **9**† | 21.2 | | 170.5 | | 51.3 | | — | | — | |
| **10**† | 21.2 | | 170.5 | | 51.4 | | — | | — | |

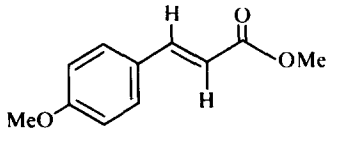
*$^{13}C$ NMR spectra were recorded on a Varian CFT-20 spectrometer operating at 20 MHz in the FT mode. The $\delta$ values are in ppm downfield from TMS.

†Data are taken from ref [4].

These non-equivalent methylene protons further coupled with the adjacent H-20 giving rise to an unresolved multiplet at $\delta$ 3.85–4.35. The coupling constants (8 and 12 Hz) of the H-3$\alpha$) signal at 4.37 indicated that the orientation of H-3 was not pure axial and that ring A assumed a twisted form (with C-2 and C-5 at the bow and stern positions) accompanied by some deformation of ring B (chair) also to avoid unfavourable *syn*-axial interaction between the 4$\beta$-Me and 10-Me groups involved in its chair conformation.




**1** R = H
**2** R = Ac



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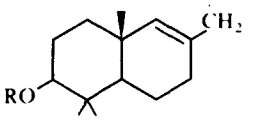
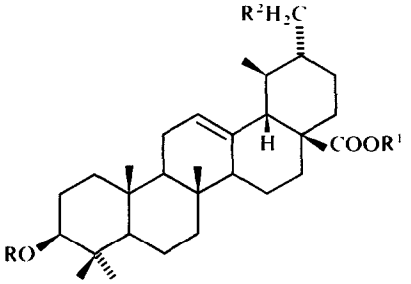
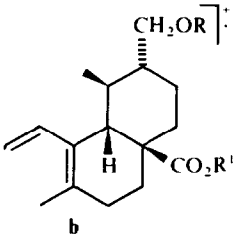


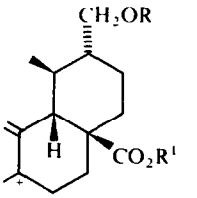
a



4 R = Ac, $R^1$ = Me, $R^2$ = OAc
5 R = $R^1$ = H, $R^2$ = OH
6 R = Ac, $R^1$ = H, $R^2$ = OAc
7 R = H, $R^1$ = Me, $R^2$ = OH
8 R = Ac, $R^1$ = $R^2$ = H
9 R = Ac, $R^1$ = Me, $R^2$ = H



b



c

Rubicoumaric acid (**1**) was hydrolysed (4% KOH–MeOH, reflux, 2 hr), methylated ($CH_2N_2$) and then acetylated ($Ac_2O$–pyridine) without prior separation. The resulting mixture of products (two spots) upon chromatography (silica gel) yielded methyl *p*-methoxy-*trans*-cinnamate (**3**), mp 90°, $C_{11}H_{12}O_3$ ($M^+$ at *m/z* 192) and a diacetate methyl ester **4**, mp 179°, $C_{35}H_{54}O_6$ ($M^+$ at *m/z* 570). The latter was shown to be a diacetyl methyl rubifolate since it underwent hydrolysis (4% KOH–MeOH, reflux, 1 hr) to give methyl rubifolate (**7**), mp 199°, $C_{31}H_{50}O_4$ ($M^+$ at *m/z* 486), $[\alpha]_D$ + 68° ($CHCl_3$), which was also obtained from rubifolic acid (**5**) by methylation with $CH_2N_2$. Rubicoumaric acid diacetate (**2**) upon hydrolysis (1% KOH–MeOH, reflux, 2 hr) afforded rubifolic acid (**5**). The latter was acetylated to yield rubifolic acid diacetate (**6**), mp 165°, $C_{34}H_{52}O_6$ ($M^+$ at *m/z* 556), $[\alpha]_D$ + 52.6° ($CHCl_3$). The IR and $^1$H NMR spectra of **3**, **4**, **6** and **7** as well as their mass spectra were fully consistent with their assigned structures.

The presence of an ursene skeleton was demonstrated by the appearance of the allylic 18 $\beta$-proton (axial) at $\delta$ 2.21, 2.28 and 2.24 as a doublet (in each case $J_{18,19}$ = 11 Hz) in the spectra of **2**, **4**, and **6** respectively as observed earlier for ursenes (e.g. in methyl ursolate H-18 is at 2.21 and $J$ = 10.7 Hz [4, 5] or 10 Hz [6]. On the contrary, the oleanenes show H-18 as a quartet with a larger $J_{18,19}$ (13.6 Hz [4, 5] or 18 Hz [6]) and $\delta$ values (H-18 of methyl oleanolate is 2.83 [4, 5] or 2.75 [6]). The smaller $J$ value indicates a twisted form of the ring E in the ursene series [4, 5].

The nature of the carbon framework of **1** and its derivatives and the location of the trisubstituted double bond were revealed by the general mass fragmentation behaviour of compounds **1**, **2**, **4**, **5** and **6**. The significant ions with appropriate mass shifts in their mass spectra, characteristic of $\Delta^{12}$-oleanenes and $\Delta^{12}$-ursenes [7], are shown in Table 1. The ions (not given in Table 1) at *m/z* 147 (8.4%) and 189 (72.5%) in the mass spectra of **1** and **2** are due to the *p*-hydroxy-*trans*-cinnamoyl and *p*-acetoxy-*trans*-cinnamoyl moieties, respectively, attached to a hydroxyl of the triterpene skeleton in an ester linkage. The site of the latter has been settled at C-3 by the ions represented by **a** (*m/z* 353 in **1** and 395 in **2**) formation of which must involve a retro-Diels–Alder type fragmentation of ring C of the $\Delta^{12}$-amyrin skeleton [7]. Ion **a** at *m/z* 249 (in **4** or **6**) and 207 (in **5**) indicates that there is only one OH or OAc group on ring A or B. The strong ion represented by **b** at *m/z* 264 (in **1** or **5**) and 306 (in **2** or **6**) and 320 (in **4**) arising from the same fragmentation when the charge remained in the more stable diene portion demonstrates that the $CH_2OH$ and COOH groups are present in ring D or E in the present triterpenoid **1** as well as in **5**. The other significant ions listed in Table 1 arose following the fragmentation pathway reported earlier for ursenes.

$^{13}$C NMR spectroscopy has been used to establish rubicoumaric acid diacetate (**2**) and diacetyl methyl rubifolate (**4**) as ursenes and not oleanenes and to elaborate their complete structures and stereochemistry. The $^{13}$C chemical shifts of **2**, **4** ursolic acid acetate (**8**), acetyl methyl ursolate (**9**), [4, 5] and acetyl methyl oleanolate (**10**) [4, 5] are shown in Table 2. The $^{13}$C signals of **2**, **4** and **8** were assigned by means of the single frequency off-resonance decoupling technique [8, 9], and by comparison of all $\delta$ values (obtained by the noise decoupling technique [8, 9] with those of the compounds included in Table 2. The $\delta$ values of **2** and **4** were almost the same for all carbons except C-28 (present as COOH in **2** and COOMe in **4**). Considerably large differences between this series of compounds and **8** and **9** of the ursene series were discernible for C-19 (*ca* −5 ppm), C-20

(*ca* −4 ppm), C-21 (*ca* −5.5 ppm) and C-30 (*ca* −46 ppm)—C-30 being present in **2** and **4** as $CH_2OR$. In the spectra of **2** and **4** C-13 and C-27 appeared at high fields and C-12, C-17, C-18 and C-22 appeared at low fields by amounts expected for ursenes (*cf.* **8** and **9**) compared to oleanenes (*cf.* **10**). These changes in $\delta$ values are attributed to the conformational change in ring E from the chair in the oleanene series to the twisted form in the ursene series, accompanied by some change in rings C and D to mitigate the strain between C-29 ($\alpha$-Me) and the proximate C-12, C-13 and C-27 ($\alpha$-Me) in the chair form of ring E [4, 5]. Quite close $\delta$ values for carbons 1–10 and 23–25 in both series having 3$\beta$-OR [4] at once indicate the same conformation of ring A in these compounds. To alleviate the *syn*-axial interaction between 4$\beta$-Me and 10-Me, and 10-Me and 8-Me, ring A may be present in a twisted form in 3$\beta$-alcohols or derivatives of both series. The $^{13}C$ shifts of the *p*-acetoxy-*trans*-cinnamoyl moiety of **2** are indicated on the partial formula **11**.

## EXPERIMENTAL

Only those IR and $^1H$ NMR peaks of the new compounds that have not been mentioned in the results and some of the mass spectral peaks not included in Table 1 are cited in this section. Mps: open capillaries, $H_2SO_4$ bath, uncorr.; IR: KBr; UV: 95% EtOH (aldehyde-free); $^1H$ NMR (80 MHz) and $^{13}C$ NMR (20 MHz): Varian CFT-20, $\delta$ values in ppm downfield from TMS; MS: 70 eV; Si gel (100–200 mesh, Gouri Chemical, Calcutta) for chromatography, unless otherwise mentioned; spots visualized in UV light and exposure to $I_2$.

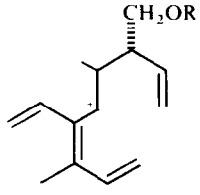
*Extraction.* Dried and powdered whole plant (5 kg) of *Rubia cordifolia* was extracted exhaustively in a Soxhlet apparatus with petrol (bp 60–80°) and $CHCl_3$ successively. The $CHCl_3$ extract was chromatographed over Si gel (60–120 mesh) using solvents and solvent mixtures of increasing polarity. The similar fractions as indicated by TLC were combined.

*Isolation of rubifolic acid* (**5**). The MeOH–$CHCl_3$ (5:95) eluate fractions from the main Si gel chromatogram afforded a solid showing two close spots on TLC ($R_f$ 0.45 and 0.5, Si gel G, $CHCl_3$–MeOH, 95:5). The latter upon repeated chromatography over Si gel yielded from the earlier MeOH–$CHCl_3$ fractions pure rubifolic acid (**5**) crystallizing from $CHCl_3$–MeOH as colourless flakes (5 mg), mp 286–287°, $[\alpha]_D$ + 58.8° (*c* 0.19, EtOH); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 2940 ($CH_2$ and CH), 1475, 1461; six peaks at 1390, 1379, 1365, 1312, 1285 and 1247 (characteristic of the ursene skeleton [10]); 1050, 982.

*Isolation of rubicoumaric acid* (**1**). The later MeOH–$CHCl_3$ (5:95) eluate fractions of the main chromatogram furnished rubicoumaric acid (**1**), which was purified to homogeneity through rechromatography, but could not be induced to crystallize and was obtained as an amorphous solid (0.4 g), mp 215°, $[\alpha]_D$ + 24.5° (*c* 0.17, $CHCl_3$); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 2968 ($CH_2$ and CH), 1680 (COOH), 1515, 1460, 1390 and 1379 ($CMe_2$), 1200, 1045, 1030; UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$): 210 (4.55), 314 (4.56); $\lambda_{min}$ 250 (3.74); $\lambda_{max}^{EtOH/OH^-}$ 210 (4.40), 368 (4.60); $\lambda_{min}^{EtOH/OH^-}$ 275 (3.84); MS *m/z* (rel. int.): 618 $[M]^+$ (1.5), 472 $[M - p(OH)C_6H_4CH{=}CHCO]^+$ (6.1), 457 $[472 - Me]^+$ (1.1), 454 (1.7), 318 (1.1), 316 (1.8), 266 (2.5), 265 (18.1), 190 (9.2), 189 (8.1), 119 (11.8), 109 (6.6), 107 (9.3), 105 (10.5), 95 (10.2), 93 (10).

*Rubicoumaric acid diacetate* (**2**). A soln of rubicoumaric acid (**1**, 150 mg) in pyridine (2 ml), was treated with $Ac_2O$ (5 ml, room temp. 18 hr). The residue, obtained after usual work-up of the reaction mixture, was chromatographed over Si gel. The $CHCl_3$–MeOH (99:1) eluate fractions afforded the diacetate **2**, crystallizing from $CHCl_3$–petrol as colourless needles, mp 205°, $[\alpha]_D$ +12° (*c* 0.083, $CHCl_3$); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 2970 ($CH_2$ and CH), 1628 (phenyl conj. C=C), 1590 (aromatic), 1495, 1440; six bands at 1400, 1380, 1359, 1308, 1270 and 1245 (characteristic of the ursene skeleton [8]); 1235 (ester C—O), 1195, 1152, 1000, 995 (*trans* CH=CH), 900, 825 (1,4-disubstituted benzene); UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$): 206 (4.30), 282 (4.37); MS *m/z* (rel. int.): 702 $[M^+]$ (1.8), 660 $[M - CH_2O]^+$ (3.7), 642 $[M - HOAc]^+$ (6.4), 557 (4), 504 (4), 452 (10), 434 (11), 405 (8), 249 (14.7), 218 (18.3), 203 (27.5), 190 (100), 189 (72.5), 175 (51.4), 173 (25.7), 164 (36.7), 161 (25.7), 159 (22.9), 147 (99), 145 (38.6), 131 (57), 121 (56.1), 119 (92.1), 107 (79.1), 105 (62.5). (Found: C, 73.12; H, 8.09. Calc. for $C_{43}H_{58}O_8$: C, 73.50; H, 8.26%).

*Diacetyl methyl rubifolate* (**4**) *and methyl p-methoxy*-trans-*cinnamate* (**3**). Rubicoumaric acid (200 mg) was refluxed with 4% KOH–MeOH soln (40 ml) on a steam bath (2 hr); the reaction mixture was neutralized with dil HCl and evapd under red. pres. in a rotary evaporator. The MeOH soln of the residue was treated with an ethereal soln of $CH_2N_2$ (excess). The product was acetylated ($Ac_2O$–pyridine, room temp). Usual work-up and chromatography of the resulting residue over Si gel gave from

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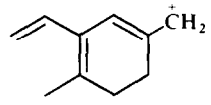
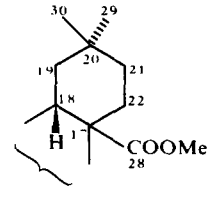


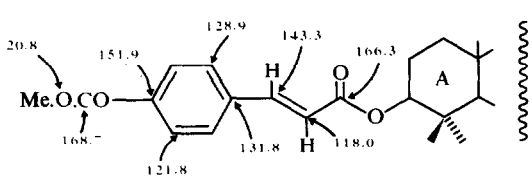

f

*m/z* 133




**10**

(The rest same as **9**)




**11**

earlier $CHCl_3$ eluates methyl *p*-methoxy-*trans*-cinnamate (**3**) (24 mg), mp 90°, $C_{11}H_{12}O_3$ ($M^+$ *m/z* 192); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1710 (conj. ester CO), 1635 (phenyl conj. C=C), 1600, 1570 and 1555 (aromatic), 1505, 1430, 1325, 1280, 1245 (CO—O), 1160, 1020, 1000, 975 (*trans* CH=CH), 925, 830 (1,4-disubstituted benzene), 810, 760; $^1H$ NMR ($CDCl_3$): $\delta$ 3.78 and 3.81 (3 H, *s* each, 4′-OMe and COOMe), 6.27 (1 H, *d*, $J_{trans}$ = 16.2 Hz, α-H), 6.86 (2 H, *d*, *J* = 8.5 Hz, H-3′ and H-5′), 7.43 (2 H, *d*, *J* = 8.7 Hz, H-2′ and H-6′), 7.62 (1 H, *d*, $J_{trans}$ = 16.3 Hz, β-H), identity was confirmed by direct comparison (IR, mmp, co-TLC) with an authentic sample.

The later $CHCl_3$ fractions afforded diacetyl methyl rubifolate (**4**) (155 mg), mp 179° ($CHCl_3$–petrol), $[\alpha]_D$ + 44° (*c* 0.064, $CHCl_3$); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 2960 (C—H), 1730 and 1720 sh (MeO.C=O and OCOMe), 1460; 1387, 1370, 1360 and 1308, 1270 infl., 1246 infl. (characteristic of the ursene skeleton [10]); 1230 (OC—O), 1192, 1020; $^1H$ NMR ($CDCl_3$): $\delta$ 0.75, 0.94 and 0.99 (3 H, *s* each, three *t*-Me's), 0.86 (6 H, *br s*, two overlapping *t*-Me's), 0.90 (3 H, *d*, *J* = 7 Hz, *sec*-Me at C-19), 2.03 (3 H, *s*, OCOMe), 2.04 (3 H, *s*, OCOMe), 2.28 (1 H, *d*, *J* = 11 Hz, H-18), 3.60 (3 H, *s*, COOMe), 3.97–4.30 (2 H, *m*, $CHCH_2OCOMe$, non-equivalent), 4.50 (1 H, *dd*, *J* = 12 and 8 Hz, H-3), 5.25 (1 H, *br s*, $W_{\frac{1}{2}}$ = 9 Hz, H-12); MS *m/z* rel int. 570 $[M]^+$ (8.9), 511 $[M-OAc]^+$ (7.3), 510 $[M-HOAc]^+$, 495 (3.8), 451 (2.7), 372 (3.6), 322 (3.5), 321 (23), 259 (5.9), 248 (4.3), 204 (6), 203 (10.5), 202 (19), 200 (11.7), 199 (8), 191 (14), 190 (37), 189 (21.7), 188 (5.5), 185 (8.6), 173 (10.6), 171 (8.4), 157 (7.3), 147 (8.7), 135 (8.9), 131 (11.2), 121 (11.1), 119 (15.0), 107 (13.8), 105 (13.8), 95 (13), 93 (13.2), 43 (38.5).

*Rubifolic acid diacetate* (**6**). Rubicoumaric acid diacetate (**2**) (100 mg) was hydrolysed by refluxing with 5% KOH–MeOH (30 ml) on a steam bath for 2 hr. Usual work-up and chromatography of the resulting residue afforded a dihydroxy acid crystallizing from $CHCl_3$–MeOH as transparent flakes (50 mg), mp 286–287°, $[\alpha]_D$ + 54.8° (*c* 0.16, EtOH). The latter was found to be identical with rubifolic acid (**5**) by direct comparison (mmp, IR, co-TLC). $Ac_2O$-pyridine treatment (room temp. 16 hr) of **5** (20 mg) followed by usual work-up afforded through chromatography over Si gel rubifolic acid diacetate (**6**) crystallizing from $CHCl_3$–petrol as needles (15 mg), mp 165°, $[\alpha]_D$ + 52.6° (*c* 0.32, $CHCl_3$): IR $\nu_{max}^{KBr}$ $cm^{-1}$: 2940 ($CH_2$ and CH), 1735 (acetate CO), 1695 (COOH); six bands at 1395, 1375, 1365, 1312, 1270 and 1259 (characteristic of the ursene skeleton [10]); 1235 (ester C—O), 1055, 1032, 982 and 969. $^1H$ NMR ($CDCl_3$): $\delta$ 0.78, 0.96 and 1.07 (3 H, *s* each, three *t*-Me's), 0.86 (6 H, *br s*, two *t*-Me's), 0.91 (3 H *d*, *J* = 7.5 Hz, *sec*-Me at C-19), 2.05 (6 H *s*, two OCOMe's), 2.24 (1 H, *d*, partial overlap with OAc signal, *J* = 11 Hz, H-18), 3.82–4.3 (2 H *m*, CH–$CH_2$–OAc, non-equivalent), 4.49 (1 H, *dd*, *J* = 10 Hz and 5 Hz, H-3), 5.25 (1 H *br s*, $W_{\frac{1}{2}}$ = 9 Hz, H-12); MS *m/z* (rel. int.): 556 $[M]^+$ (1.2), 496 $[M-HOAc]^+$ (4.1), 437 $[M-HOAc-OAc]^+$ (1.2), 262 $[b-CO_2]^+$ (7.7).

*Methyl rubifolate* (**7**). Diacetyl methyl rubifolate (**4**) (50 mg) was refluxed for 1 hr with 4% KOH–MeOH soln (40 ml). Usual work-up and chromatography of the resulting residue afforded from the 5% MeOH–$CHCl_3$ eluates methyl rubifolate (**7**), crystallizing from $CHCl_3$–MeOH in colourless globules (30 mg), mp 199°, $[\alpha]_D$ + 68° (*c* 0.175, $CHCl_3$); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (OH), 2915 (CH stretching), 1732, 1705 (ester CO), 1455 ($CMe_2$); 1390, 1379, 1360 and 1309, 1272, 1247 (characteristic of the ursene skeleton [10]); 1190, 1130, 1090, 1045, 995. $^1H$ NMR ($CDCl_3$): $\delta$ 0.75, 0.78, 0.98 (3 H, *s* each, three *t*-Me's), 0.86 (6 H, *s*, two *t*-Me's), 1.02 (3 H, *d*, *J* = 7.1 Hz, *sec*-Me at C-19), 2.27 (1 H, *d*, *J* = 10.6 Hz, H-18), 3.05–3.32 (2 H, *m*, $-CH_2-OH$), 3.60 (3 H, *s*, –COOMe), 3.65 (1 H, *m*, overlapping with OMe signal, H-3), 5.23 (1 H, *t*, *J* ≈ 3 Hz, H-12). MS *m/z* (rel. int.): 486 $[M]^+$ (2.7), 471 $[M-15]^+$ (0.6), 455 $[M-CH_2OH]^+$, (0.54), 426 $[M-COOMe-H]^+$ (2.2), 147 (10.2), 119 (18.9). Methyl rubifolate (**7**) (12 mg) was also prepared by methylation of rubifolic acid (**5**) (20 mg, obtained from **2** by hydrolysis) dissolved in MeOH (30 ml) with cooled ethereal soln of $CH_2N_2$ in excess followed by chromatography and crystallization of the resulting product.

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## REFERENCES

1. Talapatra, S. K., Patra, A. and Talapatra, B. (1978) *J. Indian Chem. Soc.* **55**, 1152.
2. Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956) *Glossary of Indian Medicinal Plants*, p. 215. C.S.I.R., New Delhi.
3. Thomson, R. H. (1971) *Naturally Occurring Quinones*. Academic Press, London.
4. Seo, S., Tomita, Y. and Tori, K. (1975) *Tetrahedron Letters* 7.
5. Seo, S., Tomita, Y. and Tori, K. (1975) *J. Chem. Soc. Chem. Commun.* 954.
6. Cheung, H. T. and Yan, T. C. (1972) *Aust. J. Chem.* **25**, 2003.
7. Budzikiewicz, H., Wilson, D. H. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
8. Levy, G. C. and Nelson, G. L. (1972) *Carbon-13 Nuclear Magnetic Resonance for Organic Chemists*. Wiley-Interscience, New York.
9. Stothers, J. B. (1972) *Carbon-13 NMR Spectroscopy*. Academic Press, New York.
10. Snatzke, G., Lampart, F. and Tschesche, R. (1962) *Tetrahedron* **18**, 1417.