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# NORCAROTENOIDS OF *REHMANNIA GLUTINOSA* VAR. *HUEICHINGENSIS**

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**Key Word Index**—*Rehmannia glutinosa* var. *hueichingensis*; Scrophulariaceae; roots; norcarotenoids; trihydroxy-β-ionone; dihydroxy-β-ionone; *sec*-hydroxyaeginetic acid; aeginetic acid; rehmapicrogenin; X-ray analysis.

**Abstract**—Two new norcarotenoids named trihydroxy-β-ionone and *sec*-hydroxyaeginetic acid, together with three known compounds, have been isolated from the steamed roots of *Rehmannia glutinosa* var. *hueichingensis*. Their structures were determined by chemical and spectral studies, and confirmed by X-ray analysis of trihydroxy-β-ionone acetate.

## INTRODUCTION

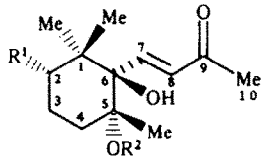
In previous papers [1–7], we reported the isolation of phenolic glycosides, iridoids, etc. from the fresh, dried and steamed roots of *Rehmannia glutinosa* Libosch. var. *hueichingensis* (Chao et Schih) Hsiao and the dried roots of *R. glutinosa* Libosch. var. *purpurea* Makino. In continuing studies on the chemical components of Rehmanniae radices, two new norcarotenoids named trihydroxy-β-ionone (**1**) and *sec*-hydroxyaeginetic acid (**3**) have been isolated from the steamed roots of *R. glutinosa* var. *hueichingensis*, together with three known compounds, dihydroxy-β-ionone (**2**) [8, 9], aeginetic acid (**4**) [10–12] and rehmapicrogenin (**5**) which was also obtained as a calcium salt (**5b**) [13].

## RESULTS AND DISCUSSION

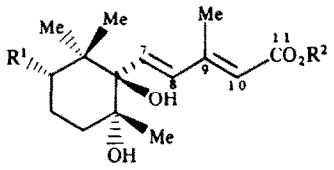
Compound **1** was isolated as needles, mp 64.5–65.5°, $[\alpha]_D$ −30.2° (MeOH). Its molecular formula $C_{13}H_{22}O_4$ was determined by high resolution FAB mass spectroscopy. The $^1$H and $^{13}$C NMR spectra of **1** resembled those of dihydroxy-β-ionone (**2**) and hence **1** was assumed to be a norcarotenoid like **2** (Tables 1 and 2). Its UV [$\lambda_{max}$: 229 nm (log ε 4.00)] and IR [$\nu_{max}$: 1672 (C=O), 1644, 1630 (C=C) cm$^{-1}$] spectral data showed the presence of an α,β-unsaturated ketone in **1**. The $^1$H NMR spectrum of **1** exhibited *trans*-olefinic proton signals at δ6.30 and 7.42 (each 1H, *d*, *J* = 16.3 Hz) and a methyl signal adjacent to the carbonyl group at δ2.32 (3H, *s*). The $^{13}$C chemical shift values for C-7 to C-10 of **1** were in good agreement with those of **2**. These spectral data indicate the existence of a 3*E*-buten-2-one side chain in **1**. The $^1$H NMR spectrum of **1** exhibited three further singlet methyl signals at δ0.89, 1.05 and 1.15 (each 3H, *s*) and its $^{13}$C NMR spectrum showed two quarternary carbon signals carrying the hydroxyl group at δ75.1 and 82.1 (each *s*). This suggestes that **1** also has a 5,6-dihydroxy-1,1,5-trimethylcyclohexyl end-group as found in **2**. However, the $^1$H and $^{13}$C signals for the cyclohexyl moiety of **1** were slightly different from those of **2**, showing the hydroxylated methine proton and carbon signals [$\delta_H$ 3.72 (1H, *dd*, *J* = 11.6, 4.0 Hz); $\delta_C$ 74.3 (*d*)]. The presence of a secondary hydroxyl group in **1** was also verified by formation of the monoacetate (**1a**) (prisms, mp 153°, FABMS *m/z* 285 $[M+H]^+$) upon ordinary acetylation of **1**. Although there are three possible positions to place the secondary hydroxyl group at from C-2 to C-4 of the cyclohexyl moiety, but the C-3 position can be at least ruled out, because the methine signal was observed as a double doublet [δ3.72 (*J* = 11.6, 4.0 Hz) in **1**; δ5.03 (*J* = 11.8, 4.3 Hz) in **1a**]. Various two-dimensional NMR experiments ($^1$H–$^1$H COSY, $^{13}$C–$^1$H COSY, NOESY, COLOC, etc.) of **1** enabled us to make a complete $^{13}$C assignment (Table 2). Comparison of the $^{13}$C chemical shift values for the cyclohexyl moiety of **1** with those of **2** indicated that the secondary hydroxyl group is located at C-2. That is, the α- (C-2) and β-carbons (C-1 and C-3) of **1** were deshielded by 37.1, 5.0 and 8.7 ppm, respectively, whereas the γ-carbons (C-4 and C-1 *gem*-dimethyls) were shielded by 0.5, 7.7 and *ca* 4 ppm, according to hydroxylation shifts [14]. The $^{13}$C NMR spectrum of **1a** gave further proof by showing typical acylation shifts: the C-2 carbon appeared at lower field by 3.7 ppm, while the adjacent C-1 and C-3 carbons resonated at higher field by 0.8 and 3.1 ppm, respectively, by comparison with those in the spectrum of **1** [3].

Regarding the configuration of the secondary hydroxyl group, the coupling constants of the methine signal [δ3.72 (*J* = 11.6, 4.0 Hz) in **1**; δ5.03 (*J* = 11.8, 4.3 Hz) in **1a**] and the Dreiding model analysis suggested that the C-2 hydroxyl group should be in the α-equatorial form, assuming that the cyclohexane ring possesses a chair conformation. In order to determine the stereo-structure of **1**, X-ray analysis was performed using a crystal of its monoacetate (**1a**). The stereoscopic view of the molecule

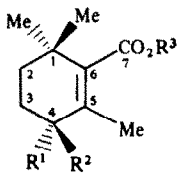
*Part 8 in the series 'Chemical and Biological Studies on Rehmanniae Radix'. For Part 7 see ref. [7].



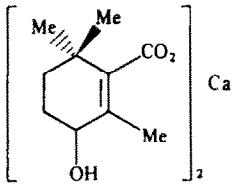
**1** $R^1$ = OH, $R^2$ = H
**1a** $R^1$ = OAc, $R^2$ = H
**2** $R^1$ = $R^2$ = H
**6** $R^1$ = H, $R^2$ = Glc



**3** $R^1$ = OH, $R^2$ = H
**3a** $R^1$ = OAc, $R^2$ = H
**3b** $R^1$ = OH, $R^2$ = Me
**4** $R^1$ = $R^2$ = H



**5** $R^1$ = $R^2$ = H or OH, $R^3$ = H
**5a** $R^1$ = $R^2$ = H or OH, $R^3$ = Me
**7** $R^1$ = *O*-Glc, $R^2$ = $R^3$ = H



**5b**

Table 1. $^1$H NMR spectral data for norcarotenoids **1**–**4** and their derivatives (500 MHz in $CD_3OD$)*

| H | 1 | 1a | 2 | 3 | 3a | 3b | 4† |
|---|---|---|---|---|---|---|---|
| 2α (eq) | | | 1.18 *dddd* (13.3, 3.4, 3.4, 1.5) | | | | 1.17 *m* |
| β (ax) | 3.72 *dd* (11.6, 4.0) | 5.03 *dd* (11.8, 4.3) | 1.69 *dddd* (13.3, 13.3, 3.7, 0.3) | 3.74 *dd* (11.8, 4.1) | 5.04 *dd* (11.8, 4.2) | 3.73 *dd* (11.8, 4.1) | [1H] 1.3–1.5 *m* |
| 3α (ax) | 1.95 *m* | 2.00 *m* | 1.91 *ddddd* (13.4, 13.3, 13.3, 3.4, 3.1) | 1.95 *m* | 2.02 *m* | 1.95 *m* | [2H] 1.6–1.9 *m* |
| β (eq) | 1.54 *m* | 1.57 *m* | 1.39 *ddddd* (13.3, 3.9, 3.7, 3.4, 3.1) | 1.53 *m* | 1.55 *m* | 1.53 *m* | [3H] |
| 4α (eq) | 1.54 *m* | 1.57 *m* | 1.48 *dddd* (13.4 3.1, 3.1, 1.5) | 1.53 *m* | 1.55 *m* | 1.53 *m* | |
| β (ax) | 1.95 *m* | 2.00 *m* | 1.80 *ddd* (13.4, 13.4, 3.9) | 1.95 *m* | 2.02 *m* | 1.95 *m* | |
| 7 | 7.42 *d* (16.3) | 7.40 *d* (16.3) | 7.43 *d* (16.3) | 6.70 *dd* (16.1, 0.3) | 6.67 *d* (16.1) | 6.72 *dd* (16.2, 0.3) | 6.71 *d* (16.0) |
| 8 | 6.30 *d* (16.3) | 6.33 *d* (16.3) | 6.34 *d* (16.3) | 6.39 *dd* (16.1, 0.8) | 6.41 *dd* (16.1, 0.7) | 6.39 *dd* (16.2, 0.8) | 6.40 *d* (16.0) |
| 10 | 2.32 *s* | 2.32 *s* | 2.31 *s* | 5.81 *br s* | 5.82 *br s* | 5.82 *br s* | 5.80 *br s* |
| $Me_2$-1 (β) | 0.89 *s* | 0.81 *s* | 0.81 *s* | 0.89 *s* | 0.81 *s* | 0.88 *s* | 0.80 *s* |
| $Me_2$-1 (α) | 1.15 *s* | 1.26 *s* | 1.24 *br s* | 1.12 *s* | 1.22 *s* | 1.11 *s* | 1.21 *s* |
| Me-5 | 1.05 s | 1.06 *s* | 1.08 *s* | 1.04 *s* | 1.05 *s* | 1.04 *s* | 1.07 *s* |
| Me-9 | — | — | — | 2.31 *d* (1.2) | 2.30 *d* (1.2) | 2.33 *d* (1.2) | 2.31 *d* (1.2) |
| $CO_2Me$ | — | — | — | — | — | 3.69 *s* | — |
| OAc | — | 2.03 *s* | — | — | 2.02 *s* | | — |

*Coupling constants (Hz) are given in parentheses. In the case of **2**, a few drops of chloroform-*d* were added to obtain a better solution.

†Measured at 200 MHz.

Table 2. $^{13}C$ NMR spectral data for norcarotenoids **1**–**5** and their derivatives (50 MHz in $CD_3OD$)*

| C | 1 | 1a† | 2 | 3 | 3a† | 3b† | 4 | 5 | 5a | 5b | Rehmapicrogenin methyl ester‡ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 44.5 | 43.7 | 39.5 | 44.8 | 43.9 | 44.8 | 39.7 | 33.8 | 33.9 | 34.0 | 33.8 |
| 2 | 74.3 | 78.0 | 37.2 | 74.5 | 78.4 | 74.6 | 37.5 | 35.4 | 35.2 | 35.9 | 35.2 |
| 3 | 27.6 | 24.5 | 18.9 | 27.7 | 24.6 | 27.7 | 19.1 | 29.7 | 29.6 | 29.8 | 29.4 |
| 4 | 36.1 | 35.6 | 36.6 | 35.9 | 35.5 | 36.0 | 36.8 | 68.4 | 68.2 | 69.8 | 68.1 |
| 5 | 75.3 | 75.2 | 75.4 | 75.5 | 75.4 | 75.6 | 75.9 | 133.7 | 136.1 | 127.6 | 135.9 |
| 6 | 82.1 | 82.1 | 80.6 | 82.2 | 82.1 | 82.3 | 80.7 | 139.2 | 137.5 | 145.0 | 137.4 |
| 7 | 152.6 | 151.5 | 153.2 | 139.5 | 138.4 | 140.0 | 140.0 | 172.7 | 170.8 | 181.1 | 170.7 |
| 8 | 131.9 | 132.3 | 131.8 | 134.5 | 135.0 | 134.4 | 134.3 | — | — | — | — |
| 9 | 201.3 | 201.1 | 201.3 | 153.7 | 152.9 | 154.2 | 153.9 | — | — | — | — |
| 10 | 27.0 | 27.2* | 27.3* | 119.7 | 120.5 | 118.9 | 119.5 | — | — | — | — |
| 11 | — | — | — | 170.7 | 171.0 | 169.3 | 170.8 | — | — | — | — |
| $Me_2$-1 | 18.0 | 19.1 | 25.7 | 18.0 | 19.0 | 18.0 | 25.8 | 28.1 | 27.9 | 28.6 | 27.8 |
| | 22.9 | 22.8 | 27.4* | 22.9 | 22.8 | 22.9 | 27.5* | 28.7 | 28.4 | 29.3 | 28.3 |
| Me-5 | 27.0 | 26.8* | 27.0* | 27.0 | 26.8 | 27.0 | 27.4* | 18.6 | 18.4 | 18.2 | 18.3 |
| Me-9 | — | — | — | 14.3 | 14.3 | 14.3 | 14.3 | — | — | — | — |
| $CO_2Me$ | — | — | — | — | 51.4 | — | — | — | 51.0 | — | ‡ |
| Me<br>\|<br>CO | — | 21.2 | — | — | 21.2 | — | — | — | — | — | — |
| | — | 172.8 | — | — | 172.9 | — | — | — | — | — | — |

*In pyridine-$d_5$ for **5** and **5a**.
†Measured at 125 MHz.
‡Data taken from ref. [12], the methyl carbon data of the carboxylmethyl group is not given.
*Interchangeable in each column.

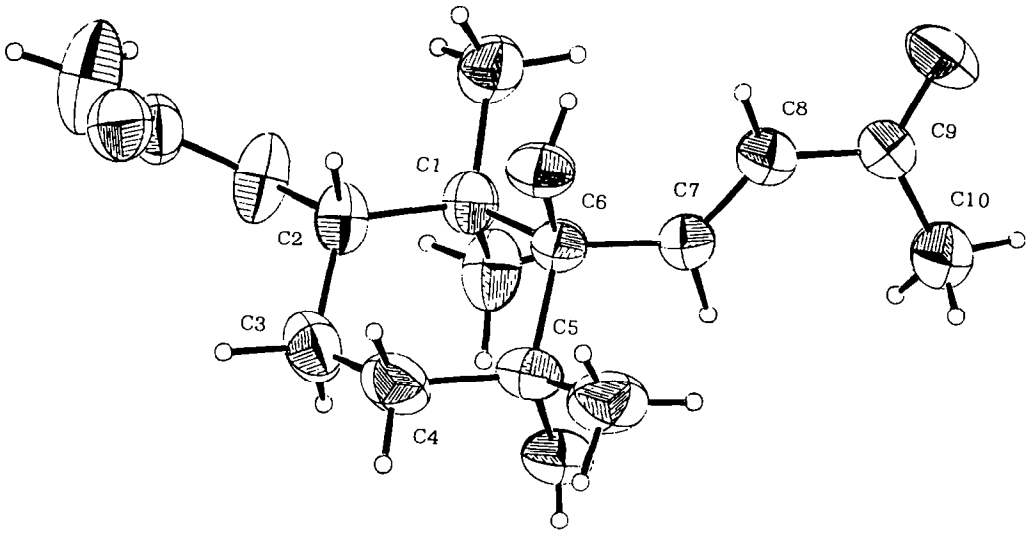



Fig. 1. X-Ray stereoscopic view of trihydroxy-β-ionone monoacetate (**1a**).

is shown in Fig. 1 and the stereochemistry is depicted on the bases of CD spectral data of **1** (see below) and also X-ray crystallographic analysis of a similar norcarotenoid glycoside, jiocarotenoside $A_1$ (see [15]). The cyclohexane ring of **1a** had a chair form ($^4C_1$) and the acetoxyl group was located at C-2 in the α-equatorial configuration.

The CD spectrum of **1** exhibited the same Cotton effects as seen in **2** or its glucoside, rehmaionoside C (**6**) [8, 13], indicating that the asymmetric centres of **1** at C-2, C-5 and C-6 are *S*, *R* and *R*, respectively. The structure of trihydroxy-β-ionone (**1**) was ultimately established as 4-[(1′*R*,2′*R*,5′*S*)-1′,2′,5′-trihydroxy-2′,6′,6′-trimethyl-1′-cyclohexyl]-3*E*-buten-2-one.

Compound **3**, needles, mp 219–220°, $[\alpha]_D$ −33.8° (MeOH), whose molecular formula $C_{15}H_{24}O_5$ was confirmed by high resolution analysis of an $[M+H]^+$ ion peak at *m/z* 285 in the FAB mass spectrum. The UV, IR, and NMR spectral data of **3** were very similar to those of aeginetic acid (**4**). The $^1H$ NMR spectrum of **3** showed three olefinic proton signals [δ5.81 (*br s*), 6.39 (*dd*,

$J = 16.1$, 0.8 Hz) and 6.70 (*dd*, $J = 16.1$, 0.3 Hz)] and one olefinic methyl signal [$\delta$2.31 (*d*, $J = 1.2$ Hz)] arising from the side chain. The $^{13}C$ chemical shift values for the side chain of **3** were superimposable with those of **4**, i.e. 3-methyl-2*E*,4*E*-pentadienoic acid. Methylation of **3** with diazomethane yielded the monomethyl ester (**3b**), needles, mp 148–150°, $[\alpha]_D$ −38.0° (MeOH), FABMS *m/z*: 299 $[M+H]^+$. With regard to the cyclohexyl end-group, the relevant $^1H$ and $^{13}C$ signals of **3** and its monoacetate (**3a**) were in good agreement with those of trihydroxy-$\beta$-ionone (**1**) and its acetate (**1a**), respectively. This indicates that the cyclohexyl end-group of **3** is the same as **1**. The CD spectrum of **3** showed similar Cotton effects to that of **1**. The structure of *sec*-hydroxyaeginetic acid (**3**) was thus determined to be 5-[(1′*R*,2′*R*,5′*S*)-1′,2′,5′-trihydroxy-2′,6′,6′-trimethyl-1′-cyclohexyl]-3-methyl-2*E*,4*E*-pentadienoic acid.

Compound **5** was obtained as needles, mp 167–168°, $[\alpha]_D$ 0° (MeOH), $C_{10}H_{16}O_3$, FABMS *m/z*: 185 $[M+H]^+$. Its $^1H$ and $^{13}C$ NMR spectral data led us to an assumption that **5** is the aglycone of rehmapicroside (**7**), which was isolated from the same plant and characterized as a norcarotenoid glucoside by Yoshikawa *et al.* [13]. On methylation with diazomethane, **5** gave the monomethyl ester (**5a**) as an oil, whose $^1H$ and $^{13}C$ NMR spectral data were coincident with those reported for the aglycone methyl ester of **7** [13]. Thus **5** was identified as rehmapicrogenin. However, the optical rotatory data of **5** and **5a** were inactive, indicating that **5** is racemic [lit., the aglycone of **7**: $[\alpha]_D$ +53.8° ($CHCl_3$)] [13]. Compound **5b** was isolated as prisms, $[\alpha]_D$ 0° (MeOH), and its $^1H$ and $^{13}C$ NMR spectral data suggested that **5b** should be the same as **5**. The melting point of **5b** was, however, found to be particularly high (mp > 300°, decomp.), and its IR spectrum exhibited an absorption band due to a carboxylate ion ($CO_2^-$) at 1536 $cm^{-1}$. Hence, **5b** seemed to be a metal salt of **5**. To ascertain which metal formed the salt, inductively coupled plasma-atomic emission spectroscopy (ICP-AES) was performed. As a result, **5b** was found to be the calcium salt.

The isolation of one norcarotenoid and four norcarotenoid glycosides have been reported from *Rehmannia* plants [8, 13]. We have isolated four further non-glycosidic norcarotenoids including two new compounds, trihydroxy-$\beta$-ionone (**1**) and *sec*-hydroxyaeginetic acid (**3**), from the steamed roots of this plant. This is the first time that rehmapicrogenin (**5**) has been isolated as a natural product. However, **5** might be formed from rehmapicroside (**7**) during the processing of the crude drug.

## EXPERIMENTAL

*General and plant material.* See refs [1, 5].

*Isolation.* Steamed roots of *R. glutinosa* var. *hueichingensis* (100 kg) were extracted with EtOH (500 l, × 2). The EtOH extract was concd to a brown mass, which was dissolved in $H_2O$ and was successively extracted with $Et_2O$, EtOAc (fr. D, 85 g) and *n*-BuOH. The $Et_2O$ extract (224 g) was further partitioned between *n*-hexane (fr. B, 93 g) and 90% MeOH aq. soln (fr. C, 112 g) [1].

Fr. C was subjected to silica gel CC (1 kg), developed with an increasing amount of MeOH in $CHCl_3$ (0:1 → 1:2), to give **4** (124 mg) and a mixt. of **3**, **5** and **5b** (*ca* 1 g). The mixt. was repeatedly applied to prep. HPLC with a prepacked CIG Si-10 column (15 mm i.d. × 30 cm), eluted with $CHCl_3$–MeOH (10:1), to yield **3** (62 mg), **5** (389 mg) and **5b** (139 mg).

Fr. D was passed through a charcoal column with $H_2O$ and then $Me_2CO$ as an eluent. The $Me_2CO$ eluate (17 g) was subjected to a silica gel CC (400 g), eluted with an increasing amount of MeOH in $CHCl_3$ (0:1 → 1:5), and was divided into 4 frs, D1–D4. Fr. D2 (1.9 g) was rechromatographed on silica gel by developing with $C_6H_6$–EtOAc (9:1) or *n*-hexane–EtOAc (1:1), to give **2** (311 mg). Fr. D3 (0.9 g) was repeatedly applied to prep. HPLC, developed with $CHCl_3$–MeOH (19:1) or $C_6H_6$–$Me_2CO$ (1:1), to afford **1** (20 mg).

*Trihydroxy-β-ionone* (**1**). Needles, mp 64.5–65.5°, $[\alpha]_D^{25}$ −30.2° (MeOH; *c* 0.75). UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$): 229 (4.00). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3450 (OH), 1672 (C=O), 1644, 1630 (C=C). $^1H$ and $^{13}C$ NMR ($CD_3OD$): see Tables 1 and 2. CD (EtOH): $[\theta]_{213}$ +15 600, $[\theta]_{224}$ −14 100, $[\theta]_{314}$ +1100. EIMS *m/z* (rel. int.): 224 $[M-18]^+$ (9), 206 (24), 142 (52), 123 (94), 109 (94), 98 (100), 95 (40), 83 (55), FDMS *m/z* 243 $[M+H]^+$. High resolution FABMS *m/z* 243.1667 $[M+H]^+$ (calc. for $C_{13}H_{23}O_4$: 243.1596).

*Trihydroxy-β-ionone monoacetate* (**1a**). Prisms, mp 153°. $^1H$ and $^{13}C$ NMR ($CD_3OD$): see Tables 1 and 2. FABMS *m/z* 285 $[M+H]^+$.

*X-Ray crystallographic analysis of compound* **1a**. The crystal size of **1a** was 0.20 × 0.25 × 0.20 mm. Unit cell dimension was obtained by least-squares refinement using 24 centered reflections for which $12° < \theta < 19°$ (graphite monochromatized Cu$K_\alpha$, $\lambda = 1.54184$ Å). Intensity data were collected at $\omega/2\theta$ scans on Enraf-Nonius CAD-4 with three check reflection at intervals of 200 reflections. Other crystal data were: $C_{15}H_{24}O_5$, orthorhombic, space group $P2_12_12_1$, $z = 4$, $a = 8.419$ (9) Å, $b = 23.510$ (2) Å, $c = 8.008$ (1) Å, $V = 1585.1$ (6) Å$^3$, $D_{calc} = 1.19$ g·$cm^{-3}$ and $\mu$ (Cu$K_\alpha$) = 6.9 $cm^{-1}$. Intensities were measured for 1776 reflections in the range $2° \leqq 2\theta \leqq 140°$ with 1510 considered as observed by the criterions $I > 3\sigma(I)$. The data were corrected for Lorents and polarization effects. No absorption correction was applied. The structure was solved by the direct-methods program Multan [16] and was refined by full-matrix least-squares, using the Enraf-Nonius SDP programs [17]. All the non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located from difference maps. The last difference Fourier map was essentially featureless with no peaks greater than 0.16 eÅ$^{-3}$. The final discrepancy index was $R = 0.048$. The ORTEP drawing is given in Fig. 1. Full crystal data are deposited at the Cambridge Crystallographic Data Center, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

*Dihydroxy-β-ionone* (**2**). Prisms, mp 103.5–104.5°. UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$): 235 (4.08). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3500, 3468 (OH), 1690 (C=O), 1628 (C=C). $^1H$ and $^{13}C$ NMR ($CD_3OD$): see Tables 1 and 2. $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$0.83, 1.24 (each 3H, *s*, $Me_2$-1), 1.14 (3H, *s*, Me-5), 1.30 (1H), 1.4–1.6 (2H), 1.6–1.9 (3H) (each *m*, H-2, H-3, H-4), 2.32 (3H, *s*, H-10), 6.36 (1H, *d*, $J = 16.1$ Hz, H-7), 7.34 (1H, *d*, $J = 16.1$ Hz, H-8). $^{13}C$ NMR (50 MHz, $CDCl_3$): $\delta$17.7 (*t*, C-3), 25.1, 26.5, 27.2, 27.5 (each *q*, C-10, $Me_2$-1 and Me-5), 36.2 (2C, *t*, C-2 and C-5), 38.5 (*s*, C-1), 74.9 (*s*, C-5), 79.5 (*s*, C-6), 130.7 (*d*, C-8), 149.6 (*d*, C-7), 198.5 (*s*, C-9). EIMS *m/z* (rel. int.): 226 $[M]^+$ (2), 208 (10), 165 (16), 141 (23), 123 (71), 109 (100), 99 (54), 85 (25), 71 (66). [lit., CD (MeOH): $[\theta]_{212}$ +14 900, $[\theta]_{242}$ −17 200, $[\theta]_{319}$ +1400] [8]. Compound **2** was identified as dihydroxy-$\beta$-ionone by direct comparison with an authentic sample [8].

*sec-Hydroxyaeginetic acid* (**3**). Needles, mp 219–220°, $[\alpha]_D^{23}$ −33.8° (MeOH; *c* 1.00). UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$): 255 (4.10). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420 (OH), 1692 (C=O), 1632, 1610 (C=C). $^1H$ and $^{13}C$ NMR ($CD_3OD$): see Tables 1 and 2. CD (EtOH): $[\theta]_{221}$ +4700, $[\theta]_{259}$ −6100. EIMS *m/z* (rel. int.): 266 $[M-18]^+$ (11), 248 (4), 184 (8), 166 (9), 153 (22), 140 (100), 122 (11), 109 (14), 101

(22). High resolution FABMS *m/z* 285.1790 $[M+H]^+$ (calc. for $C_{15}H_{25}O_5$: 285.1702).

sec-*Hydroxyaeginetic acid monoacetate* (**3a**). Amorphous powder. $^1H$ and $^{13}C$ NMR ($CD_3OD$): see Tables 1 and 2. FABMS *m/z* 349 $[M+Na]^+$.

sec-*Hydroxyaeginetic acid monomethyl ester* (**3b**). Etherial $CH_2N_2$ was added to a soln of **3** (10 mg) in MeOH (5 ml) and kept at room temp. overnight. Evapn of the reaction mixt. gave **3b** (8 mg) as needles, mp 148–150°, $[\alpha]_D^{23}$ −38.0° (MeOH; *c* 0.75). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3412 (OH), 1698 (C=O), 1634, 1614 (C=C). $^1H$ and $^{13}C$ NMR ($CD_3OD$): see Tables 1 and 2. EIMS *m/z* (rel. int.): 280 $[M-18]^+$ (11), 265 (11), 198 (15), 177 (13), 166 (46), 154 (83), 149 (24), 122 (100), 109 (35), 101 (40), 95 (88), 83 (70). FABMS *m/z* 299 $[M+H]^+$.

*Aeginetic acid* (**4**). Prisms, mp 204–205°, $[\alpha]_D^{23}$ −63.6° (MeOH; *c* 0.84). UV $\lambda_{max}^{EtOH}$ nm (log ε): 257 (4.34). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3464 (OH), 1694 (C=O), 1638, 1606 (C=C). $^1H$ and $^{13}C$ NMR ($CD_3OD$): see Tables 1 and 2. $^1H$ NMR (80 MHz, pyridine-$d_5$): δ1.18, 1.63 (each 3H, *s*, $Me_2$-1), 1.56 (3H, *s*, Me-5), 1.2–2.4 (6H in total, *m*, H-2, H-3 and H-4), 2.57 (3H, *d*, *J* = 1.1 Hz, Me-9), 6.24 (1H, *br s*, H-10), 6.94 (1H, *d*, *J* = 16.0 Hz, H-8), 7.25 (1H, *d*, *J* = 16.0 Hz, H-7). EIMS *m/z* (rel. int.): 268 $[M]^+$ (10), 250 (8), 207 (32), 141 (3), 127 (50), 113 (24), 109 (100). (Found: C, 65.59; H, 8.93. Calc. for $C_{15}H_{24}O_4 \cdot 1/3H_2O$: C, 65.68; H, 9.06%). These data were identical to those of aeginetic acid described in the literature [10–12].

*Rehmapicrogenin* (**5**). Needles, mp 167–168°, $[\alpha]_D^{24}$ 0° (MeOH; *c* 0.27). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3350 (OH), 1682 (C=O), 1650 (C=C). $^1H$ NMR (200 MHz, pyridine-$d_5$): δ1.35, 1.38 (each 3H, *s*, $Me_2$-1), 1.50, 1.85 (each 1H, *m*, H-2), 2.05 (2H, *m*, H-3), 2.25 (3H, *s*, Me-5), 4.27 (1H, *t*, *J* = 5.5 Hz, H-4). $^{13}C$ NMR (pyridine-$d_5$): see Table 2. EIMS *m/z* (rel. int.): 184.1102 $[M]^+$ (calc. for $C_{10}H_{16}O_3$: 184.1099) (17), 169 (27), 166 (17), 151 (30), 139 (77), 128 (43), 123 (78), 110 (100), 107 (52), 95 (38), 91 (43), 81 (30). FABMS *m/z* 185 $[M+H]^+$, 207 $[M+Na]^+$, 223 $[M+K]^+$.

*Rehmapicrogenin monomethyl ester* (**5a**). Etherial $CH_2N_2$ was added to a soln of **5** (13 mg) in THF (10 ml) and the whole was kept at room temp. overnight. Evapn of the reaction mixt. gave **5a** as an oil (11 mg), $[\alpha]_D^{26}$ 0° ($CHCl_3$; *c* 0.69) [lit., $[\alpha]_D^{20}$ +53.8° ($CHCl_3$)] [13]. IR $\nu_{max}^{CCl_4}$ $cm^{-1}$: 3444 (OH), 1728 (C=O), 1652 (C=C), 1222, 1062. $^1H$ NMR (500 MHz, pyridine-$d_5$): δ1.15, 1.17 (each 3H, *s*, $Me_2$-1), 1.44 (1H, *ddd*, *J* = 13.0, 8.5, 3.3 Hz, H-2), 1.76 (1H, *ddd*, *J* = 13.0, 9.6, 3.2 Hz, H-2), 2.00 (2H, *m*, H-3), 2.02 (3H, *s*, Me-5), 3.76 (3H, *s*, $CO_2Me$), 4.19 (1H, *t*, *J* = 5.6 Hz, H-4). $^1H$ NMR (200 MHz, $CDCl_3$): δ1.08, 1.10 (each 3H, *s*, $Me_2$-1), 1.35–2.05 (4H in total, *m*, H-2, H-3), 1.76 (3H, *s*, Me-5), 3.76 (3H, *s*, OMe), 3.98 (1H, *d*, *J* = 5.1 Hz, H-3). $^{13}C$ NMR (pyridine-$d_5$): see Table 2. EIMS *m/z* (rel. int.): 198.1249 $[M]^+$ (20) (calc. for $C_{11}H_{18}O_3$: 198.1256), 183 (37), 167 (25), 151 (31), 142 (100), 139 (99), 123 (80), 110 (99), 95 (36), 82 (30). Apart from the optical rotatory data, these data were identical to those of rehmapicrogenin monomethyl ester described in the literature [13].

*Rehmapicrogenin calcium salt* (**5b**). Prisms, mp >300° (decomp.), $[\alpha]_D^{27}$ 0° (MeOH; *c* 0.21). UV $\lambda_{max}^{EtOH}$ nm (log ε): 258 (2.57). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420 (OH), 1660 (C=C), 1536 ($CO_2^-$), 1395. $^1H$ NMR (200 NHz, $CD_3OD$): δ1.11, 1.15 (each 3H, *s*, $Me_2$-1), 1.35 (1H), 1.45–1.75 (2H), 1.80 (1H) (each *m*, H-2 and H-3), 1.75 (3H, *s*, Me-5), 3.86 (1H, *t*, *J* = 5.1 Hz, H-4). $^{13}C$ NMR ($CD_3OD$): see Table 2. EIMS *m/z* (rel. int.): 166 (11), 151 (7), 139 (10), 128 (6), 122 (36), 110 (9), 107 (100), 105 (34), 91 (50), 79 (33). FABMS *m/z* 183 $[C_{10}H_{15}O_3]^+$. ICP-AES conditions: plasma power, 1.3 kW; flow rate of plasma gas (Ar), 16 l $min^{-1}$; analytical wavelength (element, nm), Mg 279.55, Ca 393.37, Na 588.99, K 766.49; sample vol. 1.2951 mg. Ca, 6.37 ppm (calc. for Ca, 6.38 ppm); Mg, Na and K, 0 ppm.

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## REFERENCES

1. Sasaki, H., Nishimura, H., Morota, T., Chin, M., Mitsuhashi, H., Komatsu, Y., Maruyama, H., Tu, G., He, W. and Xiong, Y. (1989) *Planta Med.* **55**, 458.
2. Sasaki, H., Nishimura, H., Chin, M. and Mitsuhashi, H. (1989) *Phytochemistry* **28**, 875.
3. Nishimura, H., Sasaki, H., Morota, T., Chin, M. and Mitsuhashi, H. (1989) *Phytochemistry* **28**, 2705.
4. Morota, T., Sasaki, H., Nishimura, H., Sugama, K., Chin, M. and Mitsuhashi, H. (1989) *Phytochemistry* **28**, 2149.
5. Morora, T., Nishimura, H., Sasaki, H., Chin, M., Sugama, K., Katsuhara, T. and Mitsuhashi, H. (1989) *Phytochemistry* **28**, 2385.
6. Morata, T., Sasaki, H., Sugama, K., Nishimura, H., Chin, M. and Mitsuhashi, H. (1990) *Phytochemistry* **29**, 523.
7. Nishimura, H., Sasaki, H., Morota, T., Chin, M. and Mitsuhashi, H. (1990) *Phytochemistry* **29**, 3303.
8. Endo, T., Taguchi, H., Sasaki, H. and Yosioka, I. (1979) *Chem. Pharm. Bull.* **27**, 2807.
9. Eschenmoser, W., Uebelhart, P. and Eugster, C. H. (1981) *Helv. Chim. Acta* **64**, 2681.
10. Dighe, S. S. and Kulkarni, A. B. (1973) *Indian J. Chem.* **11**, 404.
11. Dighe, S. S. and Kulkarni, A. B. (1974) *Indian J. Chem.* **12**, 413.
12. Dighe, S. S., Manerikar, S. V. and Kulkarni, A. B. (1977) *Indian J. Chem.* **15B**, 546.
13. Yoshikawa, M., Fukuda, Y., Taniyama, T., Cha, B. and Kitagawa, I. (1986) *Chem. Pharm. Bull.* **34**, 2294.
14. Stothers, J. B. (1972) in *Carbon-13 NMR Spectroscopy*, pp. 163–183. Academic Press, New York.
15. Sasaki, H., Nishimura, H., Morota, T., Katsuhara, T., Chin, M. and Mitsuhashi, H. (1991) *Phytochemistry* **30**, 1639.
16. Main, P., Fiske, S. J., Hull, S. E., Lessinger, L., Germain, G., Declercq, J. P. and Woolfson, M. M. (1982) in *MULTAN* 11/82. *A System of Computer Programs for the Automatic Solution of Crystal Structures from X-Ray Diffraction Data.* University of York, U.K.
17. Frenz, B. A. (1985) in *Enraf-Nonius Structure Determination Package*, SDP/VAX V. 3.6, Enraf-Nonius, Delft, Holland.