Phytochemistry, Vol 28, No 10, pp 2705–2709, 1989
Printed in Great Britain

0031-9422/89 $3.00 + 0.00

SIX IRIDOID GLYCOSIDES FROM *REHMANNIA GLUTINOSA**

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(*Received* 16 *November* 1988)

Key Word Index—*Rehmannia glutinosa*, Scrophulariaceae, iridoids, acylated ajugols, ajugol, 8-epiloganic acid

Abstract—Six new acylated iridoids, along with 8-epiloganic acid and ajugol, have been isolated from the roots of *Rehmannia glutinosa* var *purpurea* On the basis of chemical and spectral analyses, the structures of new compounds have been established as the 6-*O*-*E*-ferulate, the 6-*O*-*Z*-ferulate, the 6-*O*-*p*-coumarate, the 6-*O*-(4″-*O*-α-L-rhamnopyranosyl) vanillate, the 6-*O*-*p*-hydroxybenzoate and the 6-*O*-vanillate of ajugol

INTRODUCTION

In the preceding paper [1], we demonstrated the presence of 10 phenethylalcohol glycosides and related compounds in the roots of *Rehmannia glutinosa* Libosch var. *purpurea* Makino In continuing the chemical examination of the constituents of this plant, we have isolated six new iridoids (**3**–**8**) having various acyl groups along with two known iridoid glucosides, 8-epiloganic acid (**1**) and ajugol (= leonuride) (**2**). This paper deals with the isolation and structural elucidation of these new compounds.

RESULTS AND DISCUSSION

Fractions B and C [1], afforded six new acylated ajugols (**3**–**8**), together with 8-epiloganic acid (**1**) [2] and ajugol (**2**) [3–5]

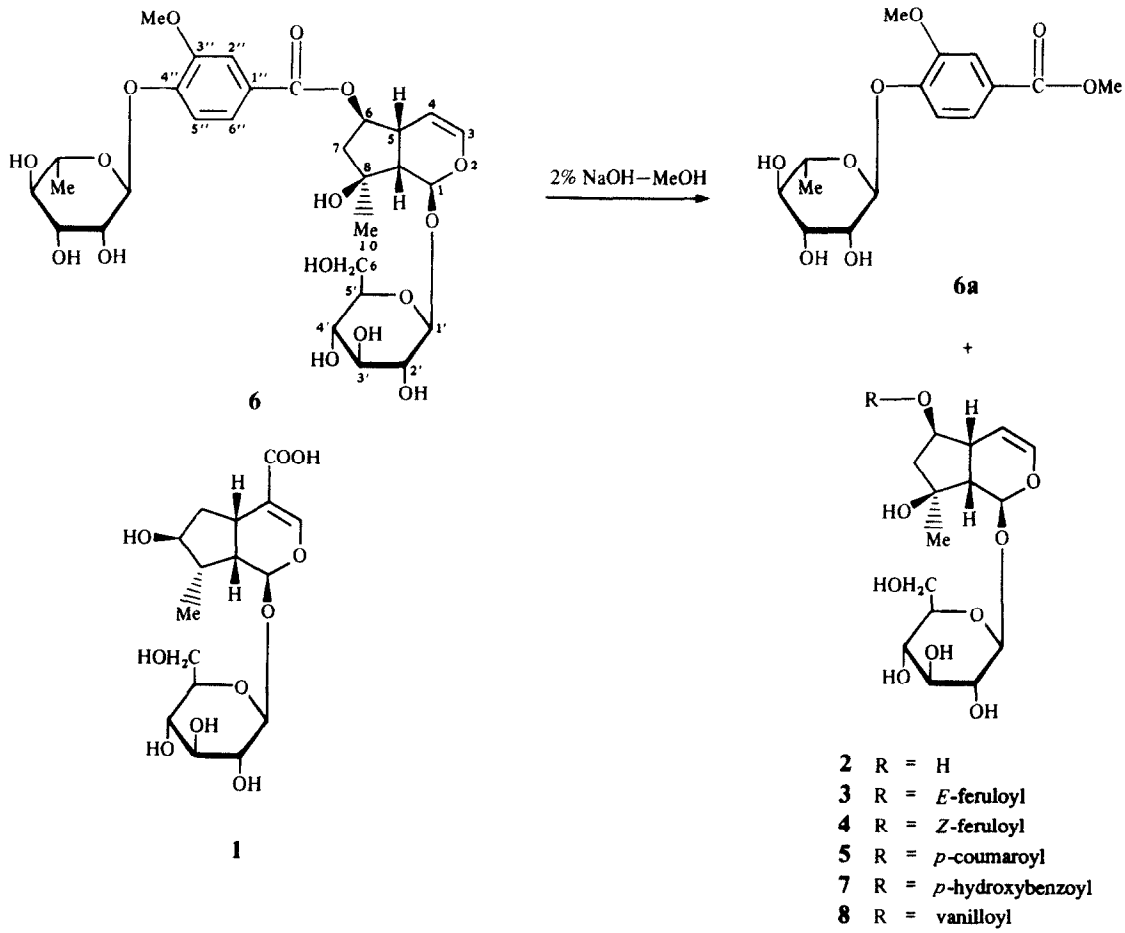
Compound **3**, $C_{25}H_{32}O_{12}\cdot 3/2H_2O$, showed an $[M]^+$ ion peak at *m/z* 524 in its FDMS It gave a reddish-violet colour with HCl [6] and a brown colour with $FeCl_3$. Furthermore, the ^{1}H NMR [CD_3OD, δ6.22 (1H, *dd*, *J* = 6.3 and 2.2 Hz, H-3 of iridoid)] and IR [ν_{max} cm^{-1}: 1690 (ester C=O)] spectral data suggested that **3** was an iridoid esterified with a phenolic acid. The ^{1}H and ^{13}C NMR spectra of **3** are very similar to those of ajugol (**2**) except for the signals arising from a *trans* feruloyl group [δ6.36 and 7.61 (each 1H, *d*, *J* = 16.0 Hz, *trans* olefin); δ6.82 (1H, *d*, *J* = 8.1 Hz), 7.05 (1H, *dd*, *J* = 8.1 and 1.7 Hz) and 7.15 (1H, *d*, *J* = 1.7 Hz) (1,3,4-trisubstituted benzene)] [1, 7]. Hydrolysis of **3** with 2% sodium hydroxide affords ferulic acid and **2**.

The ^{1}H NMR spectrum of **3** in acetone-d_6 shows a multiplet at δ4.91 (1H) and a pair of double-doublets at δ2.00 (1H, *J* = 14.2 and 4.4 Hz) and 2.23 (1H, *J* = 14.2 and 6.5 Hz). The latter signals can be readily assigned to the C-7 methylene in the ajugol moiety because of their large geminal coupling constants. The ^{1}H spin decoupling experiment indicated that the C-7 methylene signal is coupled with the multiplet signal at δ4.91, therefore this multiplet can be ascribed to the C-6 methine. The feruloyl group can be placed at C-6 of the ajugol moiety based on the fact that the C-6 methine signal of **3** is shifted downfield by 1.03 ppm when compared with that of **2** (δ3.92). The ^{13}C NMR spectral data (Table 1) of **3** also exhibits acylation shifts, i.e. the C-6 methine signal is shifted downfield (+2.0 ppm), whereas the neighbouring C-5 and C-7 carbons appear at higher field [−2.1 (C-5) and −2.3 (C-7) ppm] than those of **2** [5, 8] Accordingly, the structure of **3** is determined to be 6-*O*-*E*-feruloyl ajugol.

Compound **4** showed the same $[M]^+$ ion peak as **3**. Compound **5**, $C_{24}H_{30}O_{11}\cdot 1/2H_2O$, FABMS *m/z* 495 $[M+H]^+$, also seemed to be a similar compound to **3** and **4**. The ^{1}H NMR spectral data of **4** and **5** are closely correlated with that of **3** with respect to the presence of the ajugol moiety. In the case of **4**, *cis* coupled olefinic signals [δ5.81 and 6.85 (each 1H, *d*, *J* = 13.1 Hz)] and an aromatic ABX signal pattern [δ6.77 (*d*, *J* = 8.3 Hz), 7.76 (*d*, *J* = 2.0 Hz) and 7.13 (*dd*, *J* = 8.3 and 2.0 Hz)] together with a methoxyl signal at δ3.87 (*s*) are readily discerned. By contrast, the ^{1}H NMR spectrum of **5** shows signals due to a *trans* olefin [δ6.34 and 7.63 (each 1H, *d*, *J* = 15.9 Hz)] and a *p*-substituted aromatic ring [δ6.81 and 7.45 (each 2H, *d*, *J* = 8.7 Hz)]. These observations imply that the *trans* feruloyl group in **3** is replaced by a *cis* feruloyl group in **4** and by a *p*-coumaroyl group in **5**. Moreover, the ^{13}C chemical shifts for the carbons due to the ajugol moieties of **4** and **5** are quite consistent with those of **3**. This indicates that the acyl groups are also attached at C-6 of ajugol in both **4** and **5**. On the basis of these observations, the structures of **4** and **5** are established as 6-*O*-*Z*-feruloyl ajugol and 6-*O*-*p*-coumaroyl ajugol, respectively

Compound **6**, gave an $[M+H]^+$ ion peak at *m/z* 645 in the FABMS. The close resemblance of the ^{1}H and ^{13}C signals due to the iridoid-glucosidic residue of **6** to those observed in **3**, **4** and **5** indicate that an acyl group is located at C-6 of the ajugol moiety. However, the ^{1}H and ^{13}C NMR spectra of **6** show the presence of an additional sugar linked to the acyl moiety Alkaline hydrolysis of **6** with 2% sodium hydroxide–methanol afforded ajugol (**2**)

*Part 3 in the series 'Chemical and Biological Studies on Rehmanniae Radix' For part 2 see ref [1]



and **6a**, $C_{15}H_{20}O_8$. The ^1H and ^{13}C NMR spectra of **6a** exhibits an ABX signal pattern [δ7.21 (*d*, J = 9.0 Hz), 7.60 (*d*, J = 2.0 Hz) and 7.62 (*dd*, J = 9.0 and 2.0 Hz)], and an ester carbonyl [δ168.3 (*s*)], and a methoxyl and a carbomethoxyl signal [δ3.87 and 3.88 (each *s*)] due to the methyl vanillate moiety. In addition, ^1H signals attributable to an anomeric proton [δ5.49 (*d*, J = 2.0 Hz)] and a *sec*-methyl [δ1.21 (*d*, J = 6.4 Hz)] indicate the presence of rhamnose in **6a**. On acid hydrolysis **6a** gives rhamnose and methyl vanillate. Thus, the structure of **6a** is determined to be methyl 4-*O*-rhamnopyranosylvanillate. The α nature of the rhamnosidic linkage was shown by the coupling constant of the anomeric carbon signal ($^1J_{C1\ H1}$ = 171.5 Hz) observed in the non-decoupled ^{13}C NMR spectrum of **6a** [9]. On the basis of these results **6** is formulated as shown.

Compounds **7** and **8** showed $[M+H]^+$ ion peaks at *m/z* 469 and 499, respectively, in their FABMS. The ^1H and ^{13}C NMR spectra of **7** and **8** also contain the signals of the ajugol moiety. With regard to the acid moiety, alkaline hydrolysis of **7** and **8** affords methyl *p*-hydroxybenzoate and methyl vanillate, respectively, together with ajugol (**2**). Thus, the structures of **7** and **8** are determined to be those of the 6-*O*-*p*-hydroxybenzoate (**7**) and 6-*O*-vanillate (**8**) of ajugol.

It has been reported by other investigators that *Rehmannia glutinosa* contains diverse iridoid glycosides such as catalpol, ajugol, rehmanniosides, etc [4, 10–12]. This paper constitutes the first report of the presence of acylated ajugols.

EXPERIMENTAL

Mps uncorr. ^1H and ^{13}C NMR spectra were measured at 200 and 50.1 MHz with TMS as int. standard. TLC was conducted on precoated silica gel and cellulose plates, and spots were visualized by spraying with $FeCl_3$, dil. H_2SO_4 and aniline–hydrogen phthalate. For prep. HPLC, a CIG Si-10 column (1.5 cm i.d. × 30 cm) was employed. Plant material was purchased from Raw Medical Trading Co., Ltd.

Extraction and isolation. Fr. B (40.2 g) [1], after removal of phenethylalcohol glycosides (ionosides etc), was subjected to MCI gel CHP20P CC with a mixture of H_2O–MeOH (1.0 → 1.1) to give three further fractions, fr. B-I, B-II and B-III. Rechromatography of fr. B-I on μ Bondapak C_{18} with an increasing amount of MeOH in H_2O (1.0 → 1.1) and on silica gel with a mixture of EtOAc–MeOH–H_2O (40.2.1 → 20.3.1) afforded **1** (1.09 g) and **2** (0.46 g). Separation of Fr. B-III by μ Bondapak C_{18} CC, eluted with a mixture of H_2O–MeOH (8.2 → 6.4), Sephadex LH-20 CC with 40% MeOH aq. as well as prep. HPLC with $CHCl_3$–MeOH (8.1), gave **6** (92 mg), **7** (136 mg) and **8** (22 mg).

Similarly, fr. C [1] was repeatedly chromatographed on MCI gel CHP20P with H_2O–MeOH (9.1 → 3.7), silica gel with $CHCl_3$–MeOH (9.1 → 8.2), μ Bondapak C_{18} with H_2O–MeOH (4.1 → 1.1) and Sephadex LH-20 with EtOH, and was finally

Table 1. ^{13}C NMR spectral data for iridoids **2**–**8** (50.1 MHz, CD_3OD)

	2	3	4	5	6	7	8
C-1	93.8	93.4	93.4	93 5	93.5	93.5	93 5
3	140.4	140.9	140.9	141.0	141.1	141.1	141.1
4	105.9	104 5	104.7	104 6	104.5	104.6	104 6
5	41 3	39 2	39.1	39.3	39.4	39 4	39 4
6	78.2	80.2	80.1	80.3	80 9	80 5	80.7
7	50.0	47 7	47 9	47.9	47.8	47.9	47.9
8	79.5	79.0	79.0	79.1	79.1	79 1	79 2
9	51 8	51.5	51.5	51 6	51.7	51.7	51 7
10	25 2	26.0	25.8	26.0	26.2	26.1	26.2
Glucose							
1′	99.4	99.3	99.4	99.4	99.4	99.4	99 5
2′	74 8	74.6	74.8	74.8	74.7	74 8	74.8
3′	77.8	77.8	78.0	78.0	77.9	78 0	78 0
4′	71.7	71.6	71 7	71 7	71.7*	71 7	71 7
5′	78.0	78 0	78 2	78.1	78 1	78.2	78.2
6′	62 9	62.8	62.9	62 9	62 9	62.9	62 9
Acyl moiety							
1″		127.6	128.2	127 2	125.9	122.5	122 9
2″		111.7	115.1	131 1	117 5	116.1	113.8
3″		150.4	148.3	116.8	151.1	132 8	152 9
4″		149.2	149.4	161.2	151.2	163.5	148.7
5″		116 4	117.0	116.8	114.4	132.8	115.9
6″		124.0	126.5	131 1	124 4	116.1	125 2
α(COO)		168.8	168.0	169.0	167 5	168 0	168.0
β		115.6	115.7	115 4	—	—	—
γ		146 7	145 4	146.6	—	—	—
–OMe		56.4	56.5	—	56.6	—	56 5
				Rhamnose			
				1	100 5		
				2	71.8*		
				3	72 2*		
				4	73.7		
				5	71.0		
				6	18.0		

*Assignments may be interchanged.

subjected to prep. HPLC with $CHCl_3$–EtOH–H_2O (8:2:0.1) to give **3** (130 mg), **4** (28 mg) and **5** (16 mg).

8-Epiloganic acid (**1**). Colourless needles, mp 211–213°. $[\alpha]_D^{28}$ −63.7° (pyridine, *c* 0.21). IR ν_{max}^{KBr} cm^{-1}: 3436 (OH), 1680 (C=O), 1644 (C=C), ^{1}H NMR (pyridine-d_5): δ1.18 (3H, *d*, *J* = 7.0 Hz, H-10), 2.2–2.4 (2H, *m*, H-6, 8), 2.62 (1H, *ddd*, *J* = 13.8, 8.8 and 4.4 Hz, H-6), 3 05 (1H, *dt*, *J* = 8.5 and 3.2 Hz, H-9), 3.56 (1H, *m*, H-5), 4 0–4 3 (4H in total, *m*, H-2′,3′,4′,5′), 4.37 (1H, *dd*, *J* = 11.7 and 5.4 Hz, H-6′), 4.55 (1H, *dd*, *J* = 11.7 and 2.4 Hz, H-6′), 5.39 (1H, *d*, *J* = 7 8 Hz, H-1′), 5.91 (1H, *d*, *J* = 3.2 Hz, H-1), 7.91 (1H, *br s*, H-3); ^{13}C NMR (pyridine-d_5): δ14 2 (*q*, C-10), 30.4 (*d*, C-5), 41.1 (*t*, C-8), 42.2 (*d*, C-6), 44 5 (*d*, C-9), 62.5 (*t*, C-6′), 71.3 (*d*, C-4′), 74.4 (*d*, C-2′), 78 1 (2C, each *d*, C-7,5′), 78 3 (*d*, C-3′), 95.6 (*d*, C-1), 100.0 (*d*, C-1′), 114.2 (*s*, C-4), 150 6 (*d*, C-3), 169.3 (*s*, C-11); FDMS *m/z*: 377 $[M+H]^+$ These data were identical with those of 8-epiloganic acid described in the literature [2].

Ajugol (**2**). A white amorphous powder, $[\alpha]_D^{24}$ −172.1° (MeOH; *c* 0.53). IR ν_{max}^{KBr} cm^{-1} 3404 (OH), 1658 (C=C); ^{1}H NMR (500 MHz, CD_3OD) δ1 31 (3H, *s*, H-10), 1.79 (1H, *dd*, *J* = 13.4 and 4.7 Hz, H-7), 2.04 (1H, *dd*, *J* = 13.4 and 5.7 Hz, H-7), 2.54 (1H, *dd*, *J* = 9.6 and 2.3 Hz, H-9), 2.72 (1H, *m*, H-5), 3 20 (1H, *dd*, *J* = 9.2 and 8.0 Hz, H-2′), 3.27 (1H, *dd*, *J* = 9.7 and 8 3 Hz, H-4′), 3.30 (1H, *m*, H-5′), 3.37 (1H, *dd*, *J* = 9.2 and 8.0 Hz, H-3′), 3 66 (1H, *dd*, *J* = 11 9 and 5.7 Hz, H-6′), 3.89 (1H, *dd*, *J* = 11.9 and 2.0 Hz, H-6′), 3.92 (1H, *dt*, *J* = 5 2 and 2.9 Hz, H-6), 4.54 (1H, *d*, *J* = 7.9 Hz, H-1′), 4.85 (1H, *ddd*, *J* = 6 3, 3 2 and 0 7 Hz, H-4), 5.46 (1H, *d*, *J* = 2.3 Hz, H-1), 6.16 (1H, *ddd*, *J* = 6.3, 2.1 and 0 5 Hz, H-3); ^{13}C NMR (CD_3OD): see Table 1. Acetylation of **2** gave penta- and hexa-acetates which were identical with authentic samples in all respects (mmp, IR, TLC and ^{1}H NMR) [13].

6-O-E-Feruloyl ajugol (**3**). A white amorphous powder, $[\alpha]_D^{28}$ −147 0° (MeOH; *c* 0.33). IR ν_{max}^{KBr} cm^{-1}: 3416 (OH), 1690 (C=O), 1660 (C=C), 1598 (arom); ^{1}H NMR (CD_3OD): δ1.39 (3H, *s*, H-10), 2.01 (1H, *dd*, *J* = 14.2 and 3.9 Hz, H-7), 2.24 (1H, *dd*, *J* = 14.2 and 6 3 Hz, H-7), 2.60 (1H, *dd*, *J* = 9.3 and 2.2 Hz, H-9), 2 94 (1H, *dd*, *J* = 9.3 and 2.2 Hz, H-5), 3.2–4.0 (6H, *m*), 3.88 (3H, *s*, OMe), 4.70 (1H, *d*, *J* = 7.6 Hz, H-1′), 4.95 (2H, *m*, H-4,6), 5.52 (1H, *d*, *J* = 2.2 Hz, H-1), 6.22 (1H, *dd*, *J* = 6.3 and 2.2 Hz, H-3), 7.61 and 6.36 (each 1H, *d*, *J* = 16.0 Hz, H-β, γ), 6.82 (1H, *d*, *J* = 8.1 Hz, H-5″), 7.05 (1H, *dd*, *J* = 8.1 and 1.7 Hz, H-6″), 7.15 (1H, *d*, *J* = 1.7 Hz, H-2″); ^{1}H NMR (acetone-d_6): δ1.38 (3H, *s*, H-10), 2.00 (1H, *dd*, *J* = 14.2 and 4.4 Hz, H-7), 2.23 (1H, *dd*, *J* = 14.2 and 6.5 Hz, H-7), 2.57 (1H, *dd*, *J* = 9 3 and 2.4 Hz, H-9), 2.86 (1H, *dd*, *J* = 9.3 and 2.4 Hz, H-5), 3.2–4.2 (6H in total, *m*, H-2′, 3′, 4′, 5′, 6′), 3.92 (3H, *s*, OMe), 4.68 (1H, *d*, *J* = 7.8 Hz, H-1′), 4.91 (1H, *m*, H-6), 4.97 (1H, *dd*, *J* = 6.3 and 2 4 Hz, H-4), 5.49 (1H, *d*, *J* = 2.4 Hz, H-1), 6.21 (1H, *dd*, *J* = 6 3 and 2.2 Hz, H-3), 7.62 and 6.42 (each 1H, *d*, *J* = 15.9 Hz, H-β, γ), 6 87 (1H, *d*, *J* = 8.1 Hz, H-5″), 7.14

(1H, *dd*, $J = 8.1$ and 2.0 Hz, H-6''), 7.35 (1H, *d*, $J = 2.0$ Hz, H-2''), ^{13}C NMR (CD_3OD) see Table 1, FDMS *m/z*. 524 $[M]^+$ (Found C, 54.89, H, 6.36 $C_{25}H_{32}O_{12}$ 3/2H_2O requires C, 54.44, H, 6.40%)

Alkaline hydrolysis of **3** A soln of **3** (60 mg) in aq 2% NaOH (5 ml) was kept at room temp overnight The reaction mixture was neutralized with Amberlite IR 120B (H^+-form) resin, and was chromatographed over silica gel Elution with EtOAc–MeOH–H_2O (20 3 2) yielded ferulic acid (10 mg) and **2** (33 mg), which were identical with authentic samples in all respects (HPLC, TLC, ^{1}H NMR and IR)

6-O-Z-*Feruloyl ajugol* (**4**) A white amorphous powder, $[\alpha]_D^{28}$ −84.5° (MeOH, *c* 0.11) IR ν_{max}^{KBr} cm^{-1} 3408 (OH), 1708 (C=O), 1660 (C=C), 1628 (C=C), 1594 (arom), ^{1}H NMR (CD_3OD) δ 1.37 (3H, *s*, H-10), 1.94 (1H, *dd*, $J = 13.9$ and 4.6 Hz, H-7), 2.23 (1H, *dd*, $J = 13.9$ and 6.6 Hz, H-7), 2.52 (1H, *dd*, $J = 9.3$ and 2.4 Hz, H-9), 2.87 (1H, *dd*, $J = 9.3$ and 2.4 Hz, H-5), 3.2–4.0 (7H, *m*), 3.87 (3H, *s*, OMe), 4.66 (1H, *d*, $J = 7.8$ Hz, H-1'), 4.9–5.0 (1H, *m*, H-6), 4.98 (1H, *dd*, $J = 6.4$ and 2.4 Hz, H-4), 5.48 (1H, *d*, $J = 2.4$ Hz, H-1), 6.85 and 5.81 (each 1H, *d*, $J = 13.1$ Hz, H-β, γ), 6.20 (1H, *dd*, $J = 6.4$ and 2.2 Hz, H-3), 6.77 (1H, *d*, $J = 8.3$ Hz, H-5''), 7.13 (1H, *dd*, $J = 8.3$ and 2.0 Hz, H-6''), 7.76 (1H, *d*, $J = 2.0$ Hz, H-2''), ^{13}C NMR (CD_3OD) see Table 1, FDMS *m/z* 524 $[M]^+$

6-O-*p-Coumaroyl ajugol* (**5**) A white amorphous powder, $[\alpha]_D^{29}$ −144.9° (MeOH, *c* 0.14) IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1688 (C=O), 1632 (C=C), 1606 (arom), ^{1}H NMR (CD_3OD) δ 1.39 (3H, *s*, H-10), 1.99 (1H, *dd*, $J = 14.2$ and 3.9 Hz, H-7), 2.24 (1H, *dd*, $J = 14.2$ and 6.4 Hz, H-7), 2.58 (1H, *dd*, $J = 9.3$ and 2.4 Hz, H-9), 2.93 (1H, *dd*, $J = 9.3$ and 2.4 Hz, H-5), 3.2–4.0 (6H, *m*), 4.67 (1H, *d*, $J = 7.8$ Hz, H-1'), 4.95 (2H, *m*, H-4, 6), 5.50 (1H, *d*, $J = 2.4$ Hz, H-1), 6.22 (1H, *dd*, $J = 6.4$ and 2.2 Hz, H-3), 7.63 and 6.34 (each 1H, *d*, $J = 15.9$ Hz, H-β, γ), 6.81 (2H, *d*, $J = 8.7$ Hz, H-3'', 5''), 7.45 (2H, *d*, $J = 8.7$ Hz, H-2'', 6''), ^{13}C NMR (CD_3OD) see Table 1, FABMS *m/z* 495 $[M+H]^+$ (Found C, 57.07, H, 6.28 $C_{24}H_{30}O_{11}$ 1/2H_2O requires C, 57.25, H, 6.21%)

6-O-(4''-O-α-L-*rhamnopyranosyl*) *Vanilloyl ajugol* (**6**) A white amorphous powder, $[\alpha]_D^{28}$ −156.0° (MeOH, *c* 0.25) IR ν_{max}^{KBr} cm^{-1} 3408 (OH), 1704 (C=O), 1658 (C=C), 1600 (arom), ^{1}H NMR (CD_3OD) δ 1.22 (3H, *d*, $J = 6.1$ Hz, rham H-6), 1.41 (3H, *s*, H-10), 2.07 (1H, *dd*, $J = 14.2$ and 3.7 Hz, H-7), 2.29 (1H, *dd*, $J = 14.2$ and 6.1 Hz, H-7), 2.63 (1H, *dd*, $J = 9.2$ and 2.4 Hz, H-9), 3.01 (1H, *dd*, $J = 9.2$ and 2.2 Hz, H-5), 3.2–4.1 (10H, *m*), 3.88 (3H, *s*, OMe), 4.69 (1H, *d*, $J = 7.6$ Hz, H-1'), 5.01 (2H, *m*, H-4, 6), 5.51 (1H, *d*, $J = 1.7$ Hz, rham H-1), 5.53 (1H, *d*, $J = 2.4$ Hz, H-1), 6.24 (1H, *dd*, $J = 6.3$ and 2.2 Hz, H-3), 7.19 (1H, *d*, $J = 8.6$ Hz, H-5''), 7.61 (1H, *d*, $J = 2.0$ Hz, H-2''), 7.63 (1H, *dd*, $J = 8.6$ and 2.0 Hz, H-6''), ^{13}C NMR (CD_3OD) see Table 1, FABMS *m/z* 645 $[M+H]^+$ (Found C, 51.83, H, 6.15 $C_{29}H_{40}O_{16}$ 3/2H_2O requires C, 51.86, H, 6.45%)

Alkaline hydrolysis of **6** A soln of **6** (30 mg) in 2% NaOH–MeOH was kept at room temp overnight The reaction mixture was neutralized with Amberlite IR 120B (H^+-form) resin, and chromatographed over silica gel Elution with EtOAc–MeOH–H_2O (40 2 1) yielded **2** (18 mg) and **6a** (14 mg), colourless needles, mp 174–176°, $[\alpha]_D^{26}$ −118.0° (MeOH, *c* 0.21) IR ν_{max}^{KBr} cm^{-1} 3500 (OH), 1708 (C=O), 1600 (arom), ^{1}H NMR (CD_3OD) δ 1.21 (3H, *d*, $J = 6.4$ Hz, rham H-6), 3.46 (1H, *t*, $J = 9.5$ Hz, rham H-4), 3.70 (1H, *m*, rham H-5), 3.87 and 3.88 (each 3H, *s*, OMe and COOMe), 3.89 (1H, *dd*, $J = 9.5$ and 3.4 Hz, rham H-3), 4.07 (1H, *dd*, $J = 3.4$ and 2.0 Hz, rham H-2), 5.49 (1H, *d*, $J = 2.0$ Hz, rham H-1), 7.21 (1H, *d*, $J = 9.0$ Hz, H-5), 7.60 (1H, *d*, $J = 2.0$ Hz, H-2), 7.62 (1H, *dd*, $J = 9.0$ and 2.0 Hz, H-6), ^{13}C NMR (CD_3OD) δ 18.0 (*q*, rham C-6), 52.6 (*q*, COOMe), 56.6 (*q*, OMe), 71.1 (*d*, rham C-4), 71.9 and 72.2 (each *d*, rham C-2, 3), 73.7 (*d*, rham C-5), 100.6 (*d*, rham C-1), 114.3 (*d*, C-2), 117.6 (*d*, C-5), 124.2 (*d*, C-6), 125.7 (*s*, C-1), 151.2 (*s*, C-3), 151.4 (*s*, C-4), 168.3 (*s*, COOMe), $^1J_{C1-H1}$ (rham) 171.5 Hz, FABMS *m/z*. 351 $[M+Na]^+$ (Found C, 54.62, H, 6.20 $C_{15}H_{20}O_8$ requires C, 54.87, H, 6.14%)

Acid hydrolysis of **6a** *with* 2 M HCl A soln of **6a** (*ca* 2 mg) in 2 M HCl (0.5 ml) was heated at 70° for 5 min The reaction mixture was shaken with EtOAc (0.5 ml) In the EtOAc layer, methyl vanillate was detected and was identified with an authentic sample by co-TLC [silica gel, R_f 0.63, C_6H_6–HCO_2Et–HCO_2H (10 4 1), R_f 0.75, $CHCl_3$–MeOH (19 1)] In the aq layer, rhamnose was identified with an authentic sample by co-TLC [cellulose, R_f 0.33, *n*-BuOH–HOAc H_2O (4 1 5)]

6-O-p-*Hydroxybenzoyl ajugol* (**7**) A white amorphous powder, $[\alpha]_D^{29}$ −138.8° (MeOH, *c* 0.10) IR ν_{max}^{KBr} cm^{-1} 3416 (OH), 1690 (C=O), 1610 (arom), ^{1}H NMR (CD_3OD) δ 1.40 (3H, *s*, H-10), 2.05 (1H, *dd*, $J = 14.2$ and 4.0 Hz, H-7), 2.28 (1H, *dd*, $J = 14.2$ and 6.4 Hz, H-7), 2.61 (1H, *dd*, $J = 9.2$ and 2.4 Hz, H-9), 2.98 (1H, *dd*, $J = 9.2$ and 2.2 Hz, H-5), 3.2–4.0 (6H, *m*), 4.67 (1H, *d*, $J = 7.8$ Hz, H-1'), 5.02 (2H, *m*, H-4, 6), 5.51 (1H, *d*, $J = 2.4$ Hz, H-1), 6.23 (1H, *dd*, $J = 6.2$ and 2.3 Hz, H-3), 6.82 (2H, *d*, $J = 9.0$ Hz, H-3'', 5''), 7.90 (2H, *d*, $J = 9.0$ Hz, H-2'', 6''), ^{13}C NMR (CD_3OD) see Table 1, FABMS *m/z*. 469 $[M+H]^+$ (Found C, 55.00, H, 6.08 $C_{22}H_{28}O_{11}$ 1/2H_2O requires C, 55.34, H, 6.12%)

6-O-*Vanilloyl ajugol* (**8**) A white amorphous powder, $[\alpha]_D^{29}$ −135.2° (MeOH, *c* 0.13) IR ν_{max}^{KBr} cm^{-1} 3416 (OH), 1692 (C=O), 1660 (C=C), 1600 (arom), ^{1}H NMR (CD_3OD) δ 1.41 (3H, *s* H-10), 2.06 (1H, *dd*, $J = 14.2$ and 3.9 Hz, H-7), 2.28 (1H, *dd*, $J = 14.2$ and 6.4 Hz, H-7), 2.62 (1H, *dd*, $J = 9.3$ and 2.4 Hz, H-9), 2.99 (1H, *dd*, $J = 9.3$ and 2.2 Hz, H-5), 3.2–4.0 (6H, *m*), 3.89 (3H, *s*, OMe), 4.68 (1H, *d*, $J = 7.8$ Hz, H-1'), 5.02 (2H, *m*, H-4, 6), 5.52 (1H, *d*, $J = 2.4$ Hz, H-1), 6.23 (1H, *dd*, $J = 6.2$ and 2.3 Hz, H-3), 6.84 (1H, *d*, $J = 8.8$ Hz, H-5''), 7.57 (1H, *d*, $J = 2.0$ Hz, H-2''), 7.58 (1H, *dd*, $J = 8.8$ and 2.0 Hz, H-6''), ^{13}C NMR (CD_3OD) see Table 1, FABMS *m/z* 499 $[M]^+$ (Found C, 53.43, H, 6.00 $C_{23}H_{30}O_{12}$ H_2O requires C, 53.48, H, 6.24%)

Alkaline hydrolysis of **5**, **7** *and* **8** Each compound (2 mg) was dissolved in 2% NaOH MeOH (0.5 ml) and kept at room temp for 30 min The reaction mixture was neutralized with Amberlite IR 120B (H^+-form) resin, and then was subjected to TLC The presence of methyl *p*-coumarate and ajugol (**2**) in **5**; methyl *p*-hydroxybenzoate and **2** in **7**, methyl vanillate and **2** in **8**, was demonstrated by TLC ajugol [R_f 0.13, $CHCl_3$–MeOH–H_2O (40 10 1), R_f 0.15, EtOAc–MeOH–H_2O (20 3 2)], methyl *p*-coumarate [R_f 0.44, C_6H_6–HCO_2Et HCO_2H (10 4 1); R_f 0.56, $CHCl_3$–EtOAc–HOAc (15 5 1)], methyl *p*-hydroxybenzoate [R_f 0.39, C_6H_6–HCO_2Et–HCO_2H (10 4 1), R_f 0.49, $CHCl_3$ EtOAc–HOAc (15 5 1)], methyl vanillate [R_f 0.52, C_6H_6–HCO_2Et–HCO_2H (10 4 1), R_f 0.78, $CHCl_3$ EtOAc–HOAc (15 5 1)]

Acknowledgements—The authors are grateful to Dr K Sugama of this Laboratory for 500 MHz NMR measurement and to Miss E Yamada for elemental analyses and for MS measurements

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