IRIDOID GLUCOSIDES FROM *Penstemon diffusus*

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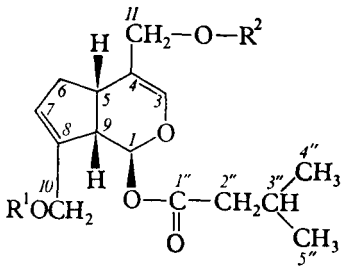
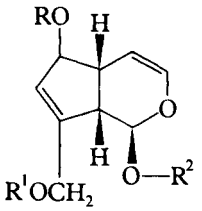
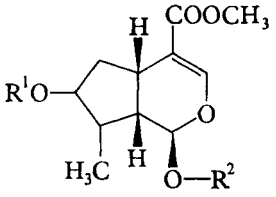
From the aerial parts of *Penstemon diffusus* DOUGL. (*Scrophulariaceae*) have been isolated three iridoid glucosides: penstemide (*I*), aucubin (*V*), and loganin (*VIII*). The identity of the compounds and their derivatives has been confirmed by spectroscopic data.

A number of rare iridoid glucosides have been reported from the aerial parts of *Penstemons* pecies[1,2]. Two of them 8-epivalerosidate[3] and penstemide (*I*) are esterified at $C_{(1)}$ with isovaleric acid and contain carbohydrate moieties attached to the $C_{(11)}$ oxymethylene group[4]. Penstemide, an antitumor agent, was found in *P. deustus* for the first time[5,6]. *P. diffusus* is the second species in which this iridoid was identified. The ethanolic extract of dried aerial parts of *P. diffusus* furnished in about 0·1% yield an oily compound *I* which was hydrolysed with β-glucosidase to give glucose and







I, $R^1 = H$; $R^2 = Glc$
II, $R^1 = Ac$; $R^2 = Glc(Ac)_4$
III, $R^1 = H$; $R^2 = H$
IV, $R^1 = Ac$; $R^2 = Ac$

V, $R^1 = H$; $R^2 = Glc$
VI, $R^1 = Ac$; $R^2 = Glc(Ac)_4$
VII, $R^1 = H$; $R^2 = H$

VIII, $R^1 = H$; $R^2 = Glc$
IX, $R^1 = Ac$; $R^2 = Glc(Ac)_4$
X, $R^1 = H$; $R^2 = H$

aglycone. The spectral properties of the compound *I* were very similar to those of penstemide[4,5,7]. The lack of standard sample of penstemide gave cause to transform compound *I* into its derivatives *II*, *III*, and *IV*. The mass spectrum of compound *I* showed a strong peak at *m/e* 444 corresponding with molecular formula $C_{21}H_{32}O_{10}$. Further peaks at *m/e* 426, 343 arose from stepwise elimination of water and isovaleric acid. Prominent peaks at *m/e* 85, 57 also indicated the presence of this acid as well

as ^{1}H NMR signals at 2·25 ppm and 0·95 ppm (Table I). Other features of ^{1}H NMR spectrum established the presence of glucose protons, doublet at 4·7 ppm ($J_{1/2}$ = = 7 Hz) and multiplet at 3·4–4·6 ppm. The remaining ^{1}H NMR data of compounds *I–IV* resembled those of penstemide. Mass spectra of minor components *V*, *VIII* isolated from the same ethanolic extract displayed fragmentation patterns characteristic for aucubin and loganin[8]. The melting points of obtained compounds *V*, *VIII* and their acetates *VI*, *IX* were also similar to those of aucubin and loganin[7].

EXPERIMENTAL

Melting points were determined on a Boetius hot stage microscope and are uncorrected. ^{1}H NMR spectra were measured at 80 or 100 MHz, using tetramethylsilane as internal standard. Mass

TABLE I

^{1}H NMR spectral data of penstemide *I* and its acetate *II*, aglycone *III*, acetate of aglycone *IV*. Run at 80 MHz in $CHCl_3$

Proton[a]	*I*[b]	*II*	*III*	*IV*
1	6·19 d (7)	5·77 d	5·85 d (7)	5·79 d (7)
3	6·46 s br	6·36 s br	6·38 s br	6·45 s br
5, 6	2·00–3·40 m	2·20–3·20	2·20–3·17 m	2·20–3·06
7	5·97 m	5·77 m	5·80 m	5·89 m
9	2·00–3·40 m	2·20–3·20	2·20–3·17 m	2·20–3·06 m
10, 11	3·70–4·60 m	4·62 m 4·17 m	4·05–4·21 s	4·49–4·64 m
1′	4·77 d (7)			
2′, 3′, 4′	3·70–4·60 m	4·75–5·56 m	—	—
5′, 6′		3·68 m 4·17 m	— —	— —
2″, 3″	2·25 m	2·22 m	2·23 m	2·22 m
4″, 5″	0·92 d (6·5)	0·97 d (6·5)	0·98 d (6·4)	0·98 d (6·5)
CH_3CO	—	1·96 s br 2·00 s 2·05 s	—	2·03 s 2·06 s

[a] Chemical shifts are given in ppm (δ scale) from tetramethylsilane as internal standard, *J* values in Hz (in parentheses); 1′–6′ glucosyl protons, 2″–5″ isovaleric acid protons; [b] run at 100 MHz in pyridine.

spectra were recorded at ionisation energy 15 and 70 eV, IR spectra in KBr discs. Silica gel 60 (70—230 mesh, Merck) was used for column chromatography. Thin layer chromatography was performed on silica gel (Merck) and developed in the following solvent systems, v/v: benzene–ethyl acetate (45 : 5) (S_1), ethyl acetate–methanol (45 : 5) (S_2), ethyl acetate–methanol–water (40 : 8 : 2) (S_3), then sprayed with vanillin reagent (vanillin 1 g, conc. hydrochloric acid 3 ml, in methanol 100 ml) or 20% sulphuric acid, and heated 5 min at 100 °C.

Plant material: *Penstemon diffusus* DOUGL. was cultivated in The Botanical Garden of the Institute of Pharmacology, Polish Academy of Sciences, Kraków, where the voucher specimen was deposited. The aerial parts of the plant were collected in August 1982.

Extraction and isolation: Dried and powdered plant material (500 g) was extracted with ethanol at room temperature and the extract evaporated under reduced pressure. The residue (50 g) was partitioned between water and chloroform. The aqueous phase was evaporated and the residue (10 g) was chromatographed on silica gel column using chloroform and mixtures chloroform with methanol (95 : 5, 90 : 10, 85 : 15) as eluents. 150 ml fractions were collected and monitored by TLC. The fractions of mixed solvents 95 : 5 were directly rechromatographed on silica gel column using chloroform–methanol gradient solvent systems, to afford an almost pure compound *I* as yellow oil. Trituration with ethyl acetate gave an amorphous powder (500 mg). Compounds *V* (40 mg) and *VIII* (20 mg) were chromatographically detected (TLC in S_2, S_3) in column fractions of mixed solvents 90 : 10, 85 : 15 and separated by preparative TLC on silica gel 60 plates, Merck, layer thickness 2 mm, developed in S_3.

Enzymatic hydrolysis: Compounds *I*, *V*, *VIII* were dissolved in 5 ml of the acetate buffer (0·5 mol l^{-1} solution of sodium acetate was adjusted to pH 5·3 with acetic acid) then β-glucosidase Sigma EC No 3.2.1.2.1. was added to the solutions and stored for 5 days at 37 °C. Aglycones were extracted with ethyl acetate. Evaporation of organic layers yielded the oily *III*, *VII*, *X*. The aqueous layers were spoted on silica gel plates together with the representative hexoses and developed in 1-butanol – acetic acid – water (4 : 1 : 1) then sprayed with 3% aqueous solution of *p*-anisidine hydrochloride and heated for 10 min at 100 °C. The spots of hydrolysis mixtures were corresponding to that of glucose.

Acetyl derivatives: Compounds *I*, *III*, *V*, *VIII* were acetylated with acetic anhydride in pyridine (1 : 1). After working up non crystalline acetates *II*, *IV* and crystalline *VI*, *IX* (methanol) were isolated. TLC was performed in S_1.

Penstemide (I): IR spectrum, KBr, cm^{-1}: 3 500—3 600, 1 750, 1 665, 1 560, 1 470, 1 290, 1 180, 1 050, 830. Mass spectrum (relative intensity), *m/e* (%): 444 (M^+, 13), 426 (M — 18, 100), 408 (M — 2 × 18, 13), 343 (M — 101, 22), 282 (M — 162, 2), 134 (53), 85 (88), 60 (17). Acetate *II*: Mass spectrum (relative intensity), *m/e* (%): 492 (M — 2 × 60 — 42, 3), 331 (24), 222 (M — 330 — 102, 5), 204 (222 — 18, 11), 162 (222 — 60, 11), 85 (35), 43 (100).

Aucubine (V): m.p. 175—178 °C (80% ethanol), (ref.[7] 180 °C). Mixed melting point with standard aucubine was undepressed. Mass spectrum (relative intensity), *m/e* (%): M^+ (—), 328 (M—18, 1), 318 (M — 28, 1), 184 (M — 162, 25), 166 (100), 138 (40), 120 (65), 109 (26), 81 (11), 73 (36), 60 (29). Acetate *VI* m.p. 123—125°C (methanol), (ref.[7] 128°C).

Loganin (VIII): m.p. 215—217°C (80% ethanol), (ref.[7] 220°C). Mixed melting point with standard loganin was undepressed. Mass spectrum (relative intensity), *m/e* (%): M^+ (—), 228 (M — 162, 27), 210 (25), 182 (100), 157 (12), 139 (28), 81 (35), 73 (11). Acetate *IX* m.p. 138 to 140°C (methanol), (ref.[7] 141°C).

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