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SHORT COMMUNICATION

A NEW IRIDOID PRECURSOR OF GENTIOPICROSIDE

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Abstract—Two new iridoids have been isolated from some *Gentiana* species. One of them, named gentioside, acts as an effective precursor of gentiopicroside (II). Its structure (V) comprising features of gentiopicroside and loganin (III) is given. The structure of the second iridoid is probably of the aucubin type.

INTRODUCTION

IN A RECENT communication[1] we described the results of some tracer experiments supporting the suggestion that gentioflavine (I), a new alkaloid from *Gentiana*, is a key intermediate in the biosynthesis of *Gentiana* alkaloids.[2] We also established that gentioflavine is in turn derived from geraniol via gentiopicroside (II).[3] Recently loganin (III) has been shown to be a precursor of gentiopicroside.[4]

The present paper describes the results of an investigation of the iridoid constituents of three *Gentiana* species (*G. asclepiadea*, *G. lutea* and *G. punctata*), compounds which are possibly intermediates in the biosynthesis of gentiopicroside in these plants.

RESULTS AND DISCUSSION

Methanol extract from fresh roots was freed from sugars. Chromatography on active charcoal and silica gel yielded three iridoid glycosides. One of them was identified as gentiopicroside, and the other two, the iridoids A and B, were unknown.

Tracer experiments were carried out in order to determine their biogenetic relationships to gentiopicroside. During a short term incubation of *G. asclepiadea* plants in $^{14}CO_2$, the radioactivity appeared in the iridoid B (both in its aglycone and sugar moieties) much earlier than in gentiopicroside, as illustrated in Table 1.

Iridoid B—we shall name it gentioside—was isolated, purified to constant activity and administered to *G. asclepiadea* plants. After 24 hr, active gentiopicroside was isolated. Its activity corresponded to 2·2% incorporation of gentioside. Obviously, gentioside is an intermediate in the biosynthesis of gentiopicroside.

Gentioside was hydrolysed by boiling in HCl; the aglycone, like all iridoid aglycones, changed to a dark resin while the carbohydrate component was identified as D-glucose.

Acetylation of gentioside yielded the acetate, m.p. 191–192°, $[\alpha]_D^{20°}$ −102°, corresponding to the acetate of the known iridoid swertiamarine.[5] But in all other properties, this substance differed from swertiamarine acetate and possessed the formula $C_{29}H_{36}O_{17}$.

[1] N. MAREKOV, M. ARNAUDOV and S. POPOV, *Compt. Rend. Acad. Bulgare Sci.* **23**, 81 (1970).
[2] N. MAREKOV and S. POPOV, *Tetrahedron* **24**, 1323 (1968).
[3] N. MAREKOV, L. MONDESHKY and M. ARNAUDOV, *Compt. Rend. Acad. Bulgare Sci.* **23**, 803 (1970).
[4] D. GROGER and P. SIMCHEN, *Z. Naturforsch.* **24b**, 356 (1969).

TABLE 1. LABELLING OF THE IRIDOIDS IN $^{14}CO_2$ ATMOSPHERE

Exposure (hr)	Isolated			
	Gentioside (mg; dis/min/mM)	Gentiopicroside (mg; dis/min/mM)	Gentioside aglycone (dis/min/mM)	Gentiopicroside aglycone (dis/min/mM)
4	6; $1{\cdot}2 \times 10^3$	95; 3×10^2	5×10^2	—
8	15; $3{\cdot}7 \times 10^3$	202; $1{\cdot}3 \times 10^3$	9×10^2	—
24	20; $7{\cdot}2 \times 10^3$	408; $1{\cdot}9 \times 10^3$	$1{\cdot}2 \times 10^3$	$8{\cdot}1 \times 10^2$

The NMR integral curve corresponded to six acetate groups, indicating the presence of two hydroxyl groups in the aglycone, while swertiamarine aglycone possesses only one. Valuable information about its structure was obtained from the UV spectrum. It showed maxima at 209, 219, 243, 251 and 269 nm, very similar to gentiopicroside (212, 218, 243, 252 and 270 nm) and this was strong evidence for the presence of the gentiopicroside chromophore (IV) in the molecule of gentioside. The structural similarity between gentiopicroside and gentioside was confirmed by the IR spectra, showing bands at 1715 cm^{-1} (conjugated ester or lactone carbonyl), 1615 cm^{-1} (conjugated double bond), 3000–3500 cm^{-1} (associated hydroxyl group). The band at 3080 cm^{-1} in the IR spectrum of gentiopicroside acetate, characteristic for a vinyl group, was absent in the IR spectrum of gentioside acetate. The NMR spectrum of gentioside acetate also confirmed the presence of moiety (IV) in the structure of gentioside. It showed signals at 3·75δ, 5·60 δ and 7·40 δ. The first and the last signals were identical with the signals for an α,β-unsaturated methoxycarbonyl group and for the olefinic proton at C3 respectively of the iridoids gentiopicroside, loganin and verbenalin. The doublet at 5·60 δ was the same as the signal for olefinic proton at C6 of gentiopicroside.

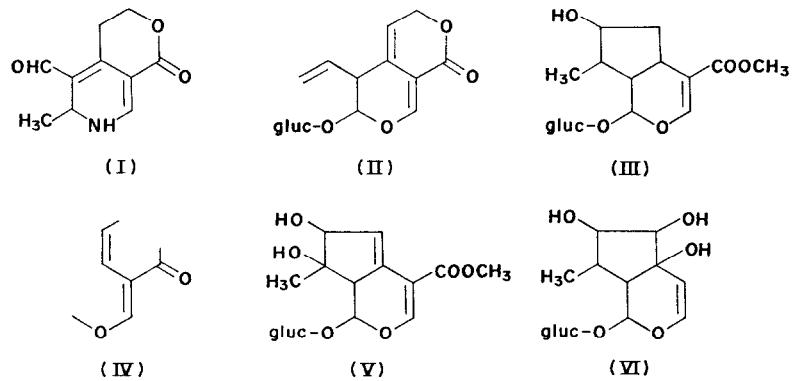
The two hydroxyl groups must be present in the remaining part of the molecule. The singlet at 1·26 δ indicated the presence of a methyl group attached to a carbon devoid of protons, similar to the methyl group of 8-hydroxyloganin (1·18 δ)[6] and of lamiol (1·17 δ).[7] The observed paramagnetic shift can be assigned to the neighbouring double bond of gentioside, absent in the mentioned two iridoids. Furthermore, these data indicated that one hydroxyl group was at C8. The second one would be situated either at C7 or C9. The presence of signals from protons at C1 and C9, as well as the comparatively easy acetylation of the hydroxyl groups of gentioside, made position 7, as in loganin, lamiide and antirride, the more probable one.

On the above basis, gentioside can be assigned structure (V), i.e. it is 8-hydroxy-5,6-dehydrologanin.

Biogenetically structure (V), comprising structural elements both of loganin and gentiopicroside, is acceptable as an intermediate stage in the biological conversion of loganin to gentiopicroside. A biological oxidation of loganin, leading to the formation of a hydroxyl group at C8 renders the C7–C8 bond more liable to cleavage.

Iridoid A yielded a pentaacetate, m.p. 198–199°, $[\alpha]_D^{20°}$ + 10°. Its UV absorption at 218 nm, IR peak at 1650 cm^{-1} and NMR singlets at 1·24 δ (methyl group), 6·45 δ (olefinic

[5] T. KUBOTA and Y. TOMITA, *Chem. & Ind.* 229 (1958).
[6] H. INOUYE, T. YOSHIDA and S. TOBITA, *Tetrahedron Letters* 2945 (1968).
[7] M. SCARPATI and M. GUISO, *Tetrahedron* **23**, 4709 (1967).



proton at C3), as well as the absence of signals at 3·70–3·80 δ (α,β-unsaturated methoxyt carbonyl group) are in agreement with a tentative structure of the aucubin type (VI), buhaving three hydroxyl groups as in charpagide.

EXPERIMENTAL

Isolation of iridoids. The roots of *Gentiana punctata* (3 kg) were extracted with methanol. After concentration to *ca.* 100 ml, the solution was diluted with 100 ml H_2O, filtered through 400 g of neutral alumina and the first 250 ml eluate collected. It was chromatographed on 600 g of active charcoal, eluted consecutively with 3 l. H_2O, 2 l. H_2O–EtOH (9:1), 2 l. H_2O–EtOH (7:3), 2 l. H_2O–EtOH (1:1) and finally with 2 l. boiling EtOH. The last two eluates as well as the hot EtOH extract contained the iridoids and were chromatographed on silica gel collecting 100 ml fractions.

The H_2O–EtOH (7:3) eluate was chromatographed on 420 g silica gel eluted with 3 l. $CHCl_3$–MeOH (3:1) and 3 l. $CHCl_3$–MeOH (2:1). Fractions 40–46 contained 240 mg of iridoid A. Acetylation with Ac_2O–pyridine afforded the acetate, m.p. 198–199° (EtOH), $[\alpha]_D^{20°}$ + 10° ($CHCl_3$). (Found: C, 53·23; H, 5·74. $C_{29}H_{36}O_{17}$ required: C, 53·05; H, 5·48%.)

The H_2O–EtOH (1:1) eluate was chromatographed on 600 g silica gel, eluted with 4 l. $CHCl_3$–MeOH (3:1) and 3 l. $CHCl_3$–MeOH (2:1). The fractions 55–63 consisted predominantly of gentioside (500 mg). Acetylation yielded the acetate, m.p. 191–192° (EtOH), $[\alpha]_D^{20°}$ −102° ($CHCl_3$).

The hot ethanol extract was chromatographed on 350 g silica gel, eluted with $CHCl_3$–MeOH (3:1). Fractions 3–12 contained 3·1 g gentiopicroside.

Feeding experiments. Three *Gentiana asclepiadea* plants were incubated for 4, 8, and 24 hr in an atmosphere of $^{14}CO_2$ (0·5 mc). From the plant material (4 hr incubation—107 g, 8 hr—217 g and 24 hr—370 g) gentioside and gentiopicroside were isolated as described (yields and activities shown in the Table). The glycosides were hydrolysed by 2 hr by boiling in HCl (1:1) and the aglycones extracted with $CHCl_3$ (yields and activities shown in Table 1).

Stems of the same plant (8 g) were put in 1 ml solution of gentioside (6 mg, spec. act. $1·2 \times 10^6$ dis/min/mM). After 24 hr the plant material was extracted with methanol, the extract concentrated to 50 ml filtered through neutral alumina. Paper chromatography in *n*-BuOH–HOAc–H_2O (63:10:27), afforded 10 mg of gentiopicroside (spec. act. $2·8 \times 10^4$ dis/min/mM) and 6 mg of gentioside (spec. act. $4·8 \times 10^4$ dis/min/mM). Hydrolysis of gentiopicroside yielded the aglycone (spec. act. $7·9 \times 10^3$ dis/min/mM).