A NEW FLAVONE GLYCOSIDE FROM SIDERITIS ROMANA L.

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From the aerial part of Sideritis romana (Labiatae) a new flavone trioside was isolated and elucidated as chrysoeriol-7-β-glucosyl-β-1→2-glucosyl-β-1→2-glucose(I)

We report on isolation and structure determination of a new glycoside from air dried aerial part of Sideritis romana L. collected on Mount Pellegrino (Sicily).

The glycoside (I), $C_{34}H_{42}O_{21}$, was isolated from ethyl acetate and ethanol extracts: m.p.235-240° (from MeOH-H_2O); uv (EtOH) λ_{max} (log ε) 248 (3.80), 268 (3.72), 347 (3.88) nm; nmr (60 MHz, DMSO-d_6) δ 3.85 (s, OCH_3), 2.90-5.50 (complex pattern), 6.30-7.70 (6 aromatic protons). The nmr spectrum (60 MHz,$CDCl_3$) of its acetate ($C_{58}H_{66}O_{33}$, m.p.139-140°) showed one aromatic methoxy-group (δ 3.90) and 12 acetoxy-groups (δ 1.90-2.5); the molecular ion is not visible in mass spectrum whereas the largest ion recognizable is at m/e 948.

These data suggest the occurrence of three hexose units in the glycoside.

When hydrolized with 7% H_2SO_4 the glycoside (I) gave a $C_{16}H_{12}O_6$ compound, m.p.330-335°, identified with the known 5,7,4'-trihydroxy-3'-methoxyflavone (chrysoeriol) (II) (lit.[2],325-330°); m/e 300 (M^+); uv (MeOH) λ_{max} (log ε) 246 (4.26), 269 (4.22), 347 (4.35) with a bathochromic shift of band I (58 nm) without a decrease in intensity and of band II (30 nm) in the presence of NaOAc[3]; bathochromic shift of band I (39 nm) in the presence of $AlCl_3$/HCl; nmr (60 MHz,DMSO-d_6) δ 3.85 (s, OCH_3), 10.0 (s, 2 OH), 12.85 (s, OH chelate).

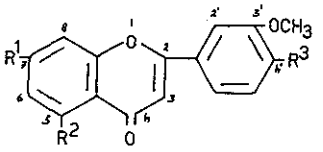
Methylation of (II) with diazometane in metanol-ether solution, gave 5-hydroxy-7,3',4'-trimethoxyflavone (III), m.p. 161-162° (from MeOH)[4]; uv (EtOH) λ_{max} (log ε) 246 (4.52), 272 (4.49), 340 (4.58) nm with a bathochromic shift of band I (16 nm) in the presence of $AlCl_3$/HCl; nmr (60 MHz,DMSO-d_6) δ 3.86 (s, 3 OCH_3), 12.85 (s, chelate OH), 7.66 (dd, J_o 9.0 Hz, J_m 2.3 Hz, 6'-H), 7.51 (d, J_m 2.3 Hz,2'-H), 7.04 (d, J_o 9.0 Hz, 5'-H), 6.70 (d, J_m 2.3 Hz, 8-H), 6.29 (d, J_m 2.3 Hz, 6-H), 6.82 (s, 3-H).

On the contrary, methylation of (II) with $(CH_3)_2SO_4$ and K_2CO_3 in acetone solution afforded 5,7,3',4'-tetramethoxyflavone (IV), m.p. 191-192° (from MeOH)[5]; uv (EtOH) λ_{max} (log ε) 240 (4.55), 263 (5.45), 330 (4.57); nmr (60 MHz,DMSO-d_6) δ 3.80 and 3.83 (2s, 2 OCH_3), 3.86 (s, 2 OCH_3), 6.69 (s, 3-H), 6.43 (d, J_m2.3 Hz, 6-H), 6.79 (d, J_m 2.3 Hz, 8-H), 7.03 (d, J_o 9.0 Hz, 5'-H), 7.48 (d, J_m 2.3 Hz, 2'-H), 7.60 (dd, J_o 9.0 Hz, J_m 2.3 Hz, 6'-H).

For direct comparison, compound (IV) was synthetized from 2-hydroxy-4,6,3',4'-tetramethoxychalcone by SeO_2 oxidation.

Permethylation of (I) by Hakomoris' method[6] gave a syrup which on hydrolysis with 7% H_2SO_4 afforded 7-hydroxy-5,3',4'-trimethoxyflavone (V), m.p. 285-286°[5]; m/e 328 (M^+); uv (EtOH) λ_{max} (log ε) 239 (4.49), 264 (4.39), 334 (4.52) nm, with bathochromic shift (30 nm) of band II in the presence of NaOAc; nmr (60 MHz-DMSO-d_6) δ3.82, 3.85 and 3.86 (s, 3 OCH_3), 6.57 (s, 3-H), 6.27 (d, J_m 2.3 Hz, 6-H), 6.45 (d, J_m 2.3 Hz, 8-H), 6.95 (d, J_o 9.0 Hz, 5'-H), 7.40 (d, J_m 2.2 Hz, 2'-H), 7.48 (dd, J_o 9.0 Hz, J_m 2.2 Hz, 6'-H) and a mixture of methylated sugars (VI).

The sugars must therefore be exclusively attached through the position 7



(II) $R^1=R^2=R^3=OH$

(III) $R^1=R^3=OCH_3$; $R^2=OH$

(IV) $R^1=R^2=R^3=OCH_3$

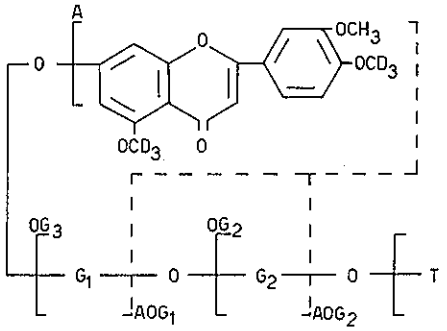
(V) $R^1=OH$; $R^2=R^3=OCH_3$

Enzymatic hydrolysis with emulsin (β-glucosidase) of the glucoside (I) dissolved in pH 5 buffer (aqueous 0.5 M NaOAc solution adjusted to pH 5 with HOAc) gave chrysoeriol (II) and only D-glucose (identified by TLC and nmr of the acetate), thus indicating a β anomeric configuration.

Mass spectra of derivatives of the glucoside (I) gave informations on the sequence and the position of the interglycosidic linkages.

From the mass spectrum of perdeuteriomethylated glycoside (VII), obtained by Hakomoris' method with CD_3I, we report selected fragments, interpreted in terms of the symbols indicated in formula (VII); inten-

sities relative to base peak at m/e 334 are in brackets.



Selected fragments from the m.s. of (VII)

Fragment symbol	m/e values	No.of OCD_3 groups	Fragment symbol	m/e values	No.of OCD_3 groups
M^+-CH_3	975 (0.5)	12	OG_3	657 (0.4)	10
AOG_2+H	744 (0.8)	8	OG_2	444 (1.5)	7
AOG_1+H	531 (2.0)	5	OG_2-CD_3OH	409 (50.0)	6
A+OH	334 (100)	2	T	231 (35.0)	4
A	317 (71.0)	2	$T-CD_3OH$	196 (71.0)	3
			$T-2CD_3OH$	161 (71.0)	2

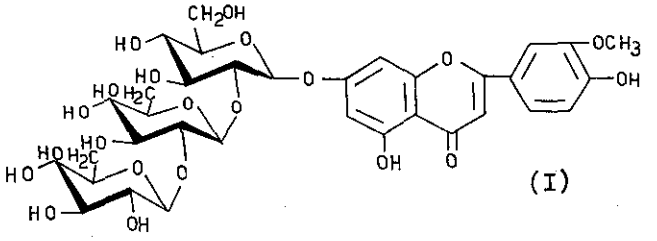
Retention of charge on the aglycone containing part of the molecule leads to the sequence of the ions AOG_2 and AOG_1.

A weak trisaccaride ion OG_3, a weak disaccaride fragment OG_2 and a strong OG_2-CD_3OH are observed. Cleavage between the glycoside oxygen and the terminal glucose unit leads to the strong fragment T; the T- series (m/e 231, 196, 161) confirms the occurrence of glucose as terminal sugar. Mass differences among OG_3

OG_2 and T confirm the presence of three glucose units in the trisaccaride residue. The lower region of m.s. is dominated by peaks due to usual fragmentation pathways of permethylated sugars[7].

G.l.c-m.s. analysis of the mixture of acetates derived from the mixture of methylated sugar of (VI) showed two peaks whose areas are in the 1:2 ratio. They were identified as 2,3,4,6-tetra-O-methyl-β-D-glucopyranose acetate (first peak) and 3,4,6-tri-O-methyl-β-D-glucopyranose diacetate (second peak)[8].

Thus the results from m.s. and g.l.c.-m.s. analysis before and after hydrolysis indicate that the original triglucoside (I) contains three moles of D-glucose joined by 1 → 2 linkages. With regard to the general observation that D-sugars occur with β-glycosidic and L-sugars with α-glycosidic linkages[9] and to the results of enzymatic hydrolysis, these data prove that the compound isolated from Sideritis romana is chrysoeriol-7-β-glucosyl-β-1→2-glucosyl-β-1→2-glucose (I)



(I)

As far we know, no glycoside of chrysoeriol (I) was found in plants of family Labiatae. Glucosyl-β-1→2-glucosyl-β-1→2-glucose occurs rarely in nature, having been found only in some species of Solanum and in Pisum sativum[10].

All the products gave satisfactory elemental analyses.

M.s. were recorded on an Hitachi-Perkin-Elmer RMU6D spectrometer, g.l.c.-m.s. on a PYE/104 device (stationary phase 3% SE 30, on Chromosorb W 80-100 mesh, programmed T 165-185°,2°/min.).

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