

A NEW FLAVONE GLYCOSIDE FROM SIDERITIS ROMANA L.Pietro Venturella\*, Aurora Bellino, and Anna PapucciInstitute of Organic Chemistry, University of Palermo  
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From the aerial part of Sideritis romana (Labiatae) a new flavone trioside was isolated and elucidated as chrysoeriol-7- $\beta$ -glucosyl- $\beta$ -1 $\rightarrow$ 2-glucosyl- $\beta$ -1 $\rightarrow$ 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<sub>2</sub>O); uv (EtOH)  $\lambda_{max}$  (log  $\epsilon$ ) 248 (3.80), 268 (3.72), 347 (3.88) nm; nmr (60 MHz, DMSO-d<sub>6</sub>)  $\delta$  3.85 (s, OCH<sub>3</sub>), 2.90-5.50 (complex pattern), 6.30-7.70 ( 6 aromatic protons). The nmr spectrum ( 60 MHz, CDCl<sub>3</sub>) of its acetate (  $C_{58}H_{66}O_{33}$ , m.p.139-140° ) showed one aromatic methoxy-group ( $\delta$  3.90) and 12 acetoxy-groups ( $\delta$  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 hydrolyzed 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.<sup>2</sup>, 325-330°); m/e 300 ( $M^+$ ); uv (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 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<sup>3</sup>; bathochromic shift of band I (39 nm) in the presence of  $AlCl_3/HCl$ ; nmr (60 MHz, DMSO- $d_6$ )  $\delta$  3.85 (s,  $OCH_3$ ), 10.0 (s, 2 OH), 12.85 (s, OH chelate).

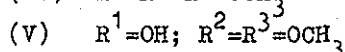
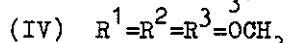
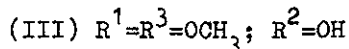
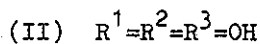
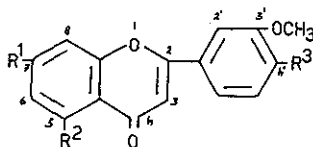
Methylation of (II) with diazometane in metanol-ether solution, gave 5-hydroxy-7,3',4'-trimethoxyflavone (III), m.p. 161-162° (from MeOH)<sup>4</sup>; uv (EtOH)  $\lambda_{max}$  (log  $\epsilon$ ) 246 (4.52), 272 (4.49), 340 (4.58) nm with a bathochromic shift of band I (16 nm) in the presence of  $AlCl_3/HCl$ ; nmr (60 MHz, DMSO- $d_6$ )  $\delta$  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)<sup>5</sup>; uv (EtOH)  $\lambda_{max}$  (log  $\epsilon$ ) 240 (4.55), 263 (5.45), 330 (4.57); nmr (60 MHz, DMSO- $d_6$ )  $\delta$  3.80 and 3.83 (2s, 2  $OCH_3$ ), 3.86 (s, 2  $OCH_3$ ), 6.69 (s, 3-H), 6.43 (d,  $J_m$  2.3 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 synthesized from 2-hydroxy-4,6,3',4'-tetramethoxychalcone by  $SeO_2$  oxidation.

Permethylation of (I) by Hakomoris' method<sup>6</sup> gave a syrup which on hydrolysis with 7% H<sub>2</sub>SO<sub>4</sub> afforded 7-hydroxy-5,3',4'-trimethoxyflavone (V), m.p. 285-286<sup>o</sup><sub>5</sub>; m/e 328 (M<sup>+</sup>); uv (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 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<sub>6</sub>)  $\delta$  3.82, 3.85 and 3.86 (s, 3 OCH<sub>3</sub>), 6.57 (s, 3-H), 6.27 (d, J<sub>m</sub> 2.3 Hz, 6-H), 6.45 (d, J<sub>m</sub> 2.3 Hz, 8-H), 6.95 (d, J<sub>o</sub> 9.0 Hz, 5'-H), 7.40 (d, J<sub>m</sub> 2.2 Hz, 2'-H), 7.48 (dd, J<sub>o</sub> 9.0 Hz, J<sub>m</sub> 2.2 Hz, 6'-H) and a mixture of methylated sugars (VI).

The sugars must therefore be exclusively attached through the position 7

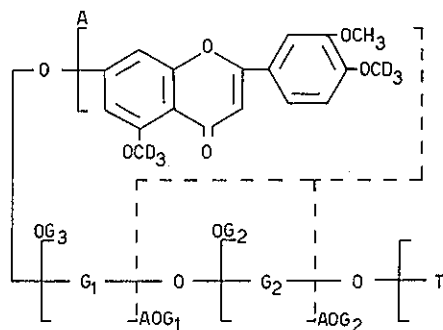


Enzymatic hydrolysis with emulsin ( $\beta$ -glucosidase) of the glucoside (I) dissolved in pH 5 buffer (aqueous 0.5 M NaOAc solution adjusted to pH 5 with HOAc) gave chrysoeriol (II) and only D-glucose (identified by TLC and nmr of the acetate), thus indicating a  $\beta$  anomeric configuration.

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<sub>3</sub>I, 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 $\text{OCD}_3$ groups	Fragment symbol	m/e values	No. of $\text{OCD}_3$ groups
$\text{M}^+ - \text{CH}_3$	975 (0.5)	12	$\text{OG}_3$	657 (0.4)	10
$\text{AOG}_2 + \text{H}$	744 (0.8)	8	$\text{OG}_2$	444 (1.5)	7
$\text{AOG}_1 + \text{H}$	531 (2.0)	5	$\text{OG}_2 - \text{CD}_3\text{OH}$	409 (50.0)	6
$\text{A} + \text{OH}$	334 (100)	2	$\text{T}$	231 (35.0)	4
$\text{A}$	317 (71.0)	2	$\text{T} - \text{CD}_3\text{OH}$	196 (71.0)	3
			$\text{T} - 2\text{CD}_3\text{OH}$	161 (71.0)	2

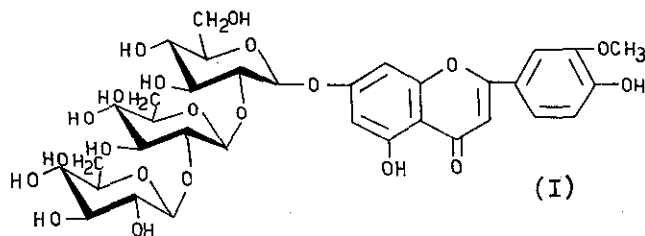
Retention of charge on the aglycone containing part of the molecule leads to the sequence of the ions  $\text{AOG}_2$  and  $\text{AOG}_1$ .

A weak trisaccharide ion  $\text{OG}_3$ , a weak disaccharide fragment  $\text{OG}_2$  and a strong  $\text{OG}_2 - \text{CD}_3\text{OH}$  are observed. Cleavage between the glycoside oxygen and the terminal glucose unit leads to the strong fragment  $\text{T}$ ; the  $\text{T}$ -series (m/e 231, 196, 161) confirms the occurrence of glucose as terminal sugar. Mass differences among  $\text{OG}_3$

OG<sub>2</sub> and T confirm the presence of three glucose units in the trisaccharide residue. The lower region of m.s. is dominated by peaks due to usual fragmentation pathways of permethylated sugars<sup>7</sup>.

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- $\beta$ -D-glucopyranose acetate (first peak) and 3,4,6-tri-O-methyl- $\beta$ -D-glucopyranose diacetate (second peak).<sup>8</sup>

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 $\rightarrow$ 2 linkages. With regard to the general observation that D-sugars occur with  $\beta$ -glycosidic and L-sugars with  $\alpha$ -glycosidic linkages<sup>9</sup> and to the results of enzymatic hydrolysis, these data prove that the compound isolated from Sideritis romana is chrysoeriol-7- $\beta$ -glucosyl- $\beta$ -1 $\rightarrow$ 2-glucosyl- $\beta$ -1 $\rightarrow$ 2-glucose (I)



As far we know, no glycoside of chrysoeriol (I) was found in plants of family Labiatae. Glucosyl- $\beta$ -1 $\rightarrow$ 2-glucosyl- $\beta$ -1 $\rightarrow$ 2-glucose occurs rarely in nature, having been found only in some species of Solanum and in Pisum sativum<sup>10</sup>.

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<sup>o</sup>, 2<sup>o</sup> /min.).

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