

CAFFEIC ACID 4-RUTINOSIDE FROM *LEONURUS CARDIACA*

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(Received 28 February 1979)

**Key Word Index** · *Leonurus cardiaca*; Labiatae; caffeic acid; 3,4-dihydroxycinnamic acid; 4-*O*-[ $\alpha$ -L-rhamnosyl-(1  $\rightarrow$  4)- $\beta$ -D-glucoside].

3-Hydroxy-4-*O*-[ $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  4)- $\beta$ -D-glucopyranosyl]cinnamic acid has been isolated for the first time from leaves and flowers of *Leonurus cardiaca* L. The aglycone was obtained by a mild hydrolysis with methanolic hydrochloric acid. The structure of the aglycone as caffeic acid was established with the aid of IR, NMR and mass spectrometric analyses. Furthermore, the identity was confirmed by TLC, mmp and superimposable IR spectrum with an authentic marker. The sugars obtained on hydrolysis were identified by PC as glucose and rhamnose. Gas chromatographic estimation of the silylated [1] sugar mixture gave a ratio of glucose: rhamnose (1:1). The inability of warm alkali to effect hydrolysis of the glycoside shows the absence of an ester linkage; moreover, the  $\text{FeCl}_3$  colour reaction indicated the presence of at least one free phenolic group in the caffeic acid moiety.

Methylation of the glycoside with dimethyl sulphate/ $\text{K}_2\text{CO}_3$  and subsequent hydrolysis with dilute HCl furnished methyl 3-methoxy-4-hydroxycinnamate as evidenced by the mp, mmp, IR identity with an authentic sample. This shows that the sugar is attached to the hydroxyl group at C-4. Exhaustive methylation of the glycoside with  $\text{MeI}/\text{Ag}_2\text{O}$  in dimethylformamide [2] followed by hydrolysis with dilute HCl gave a mixture of 2,3,4-tri-*O*-methyl-L-rhamnose and 2,3,6-tri-*O*-methyl-D-glucose identified by comparison with authentic samples. The production of these sugar derivatives proves that both glucose and rhamnose are bound in a chain to a single hydroxyl group of the aglycone, the latter being the terminal sugar. The linkage of D-glucose with the aglycone is presumed to be  $\beta$ -glycosidic by analogy with the glucoside obtained by Klostermann *et al.* [3] from flax seed and identified as methyl 4- $\beta$ -glucopyranosylcinnamate.

The isolation of this compound from *Leonurus cardiaca* L. disproves the statement of Romanowsky *et al.* [4] that this plant lacks any glycosides. A cardiological test with the glycoside on isolated guinea pig hearts did not show any remarkable effect on the activity of the heart. We thank Dr. H. Jaggy of Dr. Wilmar Schwabe Company, Karlsruhe, FRG, for conducting this test.

## EXPERIMENTAL

A crude extract of the plant *Leonurus cardiaca* L. was supplied by the firm Gebr. Guilini, Ludwigshafen, FRG. The solvent used

for extraction was  $\text{CH}_2\text{Cl}_2$ -MeOH (1:1). 500 g dry dark extract was dissolved in MeOH (3 l.) and diluted with  $\text{H}_2\text{O}$  until slight turbidity appeared. The soln was washed ( $3 \times 250$  ml) with petrol (bp 40–60°) and the glycoside was extracted with *n*-BuOH ( $5 \times 200$  ml). The BuOH soln was evapd to dryness and the residue (42 g) was examined by TLC. The mixture of glycosides was separated by column chromatography on Si gel in a  $\text{CHCl}_3$ - $\text{H}_2\text{O}$ -MeOH system [5] of gradually increasing polarity (65:10:25, 65:10:30, 65:10:35). The amount of crude glycoside obtained was 3.5 g, which was purified by a second column prepared by Sigel using  $\text{CHCl}_3$ -MeOH- $\text{H}_2\text{O}$  (64:10:1) as eluent: yield 1.6 g; mp 151–154°;  $[\alpha]_D^{25} = -5.38^\circ$  ( $c$  0.4, MeOH).

**Methyl 3,4-dihydroxycinnamate.** Mp 158–159° (yellow needles from benzene-MeOH), lit. [6] 158–160°;  $\text{C}_{10}\text{H}_{10}\text{O}_4 \cdot \frac{1}{2} \text{MeOH}$  (200); Found: C, 60.24; H, 5.07. Calc.: C, 60.00; H, 5.19%. Molecular mass peak at  $m/e = 194$ . IR (KBr)  $\text{cm}^{-1}$ : 3460 and 3280 (OH), 1675 (C=O), 1630 and 930 (C=C), 1515–1590 (aromatic).  $^1\text{H}$  NMR ( $\text{CD}_3\text{COCD}_3$ ):  $\delta$  3.67 (s, 3H), 6.2 and 7.5 (2d,  $J = 16$  Hz, olefinic protons), 6.7–7.06 (3 aryl protons), 8.1 (2 hydroxy protons).

**Methyl 3-methoxy-4-hydroxycinnamate.** Mp 62–63° ( $\text{Et}_2\text{O}$ ), lit. [6] mp 63–64°;  $\text{C}_{11}\text{H}_{12}\text{O}_4 \cdot 0.6 \text{H}_2\text{O}$  (211); Found: C, 62.37; H, 5.67. Calc. C, 62.55; H, 5.18%. Molecular mass peak at  $m/e = 208$ . IR ( $\text{CHCl}_3$ )  $\text{cm}^{-1}$ : 3510 (OH), 2980–2830 (C–H), 1700 (C=O), 1680 and 930 (C=C), 1500–1590 (aromat.).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  3.72 and 3.82 (2s, methoxy protons), 6.15 and 7.5 (2d,  $J = 16$  Hz, olefinic protons), 6.73–6.88 (3 aryl protons).

**Permethylaton of glycosides and identification of methyl sugars.** 2,3,4-Tri-*O*-methyl-L-rhamnose-anilide, mp 122–125°, lit. [7]. mp 124–126°; 2,3,6-tri-*O*-methyl-D-glucose, mp 115–118° (isopropyl ether- $\text{CHCl}_3$ ), lit. [8] mp 112–116°.

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