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SHORT COMMUNICATION

COUMESTANES IN *CICER ARIETINUM*[1]

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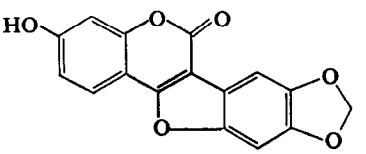
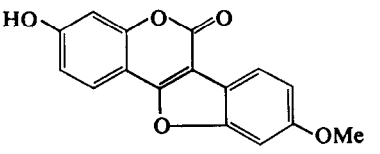
Abstract—Two coumestanes which are present in small amounts in the roots of *Cicer arietinum* were identified as medicagol and 12-*O*-methylcoumestrol by chromatographic and spectroscopic methods.

INTRODUCTION

ISOFLAVONES and coumestanes are biogenetically related.[2] The co-occurrence of isoflavones and coumestanes in a number of Papilionatae has been reported.[3] Chana germ (*Cicer arietinum* L.) contains the isoflavones biochanin A and formononetin and has been used extensively for biosynthetic studies of these and related compounds. In this paper we report the isolation and identification of medicagol[5] (I) (7-hydroxy-11,12-methylenedioxycoumestane) and 12-*O*-methylcoumestrol[4] (II) (7-hydroxy-12-methoxycoumestane) from this plant.



(I)



(II)

RESULTS

The coumestanes were isolated from the roots of 4-week-old *Cicer* plants. After enzymic hydrolysis of the glycosides by the glycosidases present in the roots, the ether extract of the aqueous phase was chromatographed on a cellulose column with aqueous acetic acid. TLC

[1] Biosynthesis of Flavonoids—XXXV; Part XXXIV: A. SUTTER and H. GRISEBACH, *Phytochem.* **8**, 101 (1969).
[2] H. ZILG and H. GRISEBACH, *Phytochem.* **7**, 1765 (1968), and earlier papers in this series.
[3] *Pachyrrizus erosus*: L. CROMBIE and D. A. WHITING, *J. Chem. Soc.* 1569 (1963); J. EISENBEISS and H. SCHMID, *Helv. Chim. Acta* **42**, 61 (1959). *Phaseolus aureus*: H. ZILG and H. GRISEBACH, *loc. cit.* **2**, addendum, *Phytochem.* **8**, 527 (1969). *Trifolium* sp.: E. M. BICKOFF, A. N. BOOTH, R. L. LYMAN, A. L. LIVINGSTON, C. R. THOMSON and F. DE EDS, *Science* **126**, 969 (1957); C. M. FRANCIS, A. J. MILLINGTON and E. T. BAILEY, *Australian J. Agric. Res.* **18**, 47 (1967). *Medicago sativa*: R. L. LYMAN, E. M. BICKOFF, A. N. BOOTH and A. L. LIVINGSTON, *Archs Biochem. Biophys.* **80**, 61 (1959).
[4] E. M. BICKOFF, A. L. LIVINGSTON, S. C. WITT, R. E. LUNDIN and R. R. SPENCER, *J. Agr. Food Chem.* **13**, 597 (1965).
[5] A. L. LIVINGSTON, S. C. WITT, R. E. LUNDIN and E. M. BICKOFF, *J. Org. Chem.* **30**, 2533 (1965).

of the eluate with 20% acetic acid indicated the presence of small amounts of 12-*O*-methylcoumestrol and/or medicagol (Table 1). After further purification by TLC the coumestane-zone had an absorption maximum at 343 nm with additional maxima at 311 and 303 nm, which indicate the presence of both I[5] and II.[6] The λ_{max} at 343 nm undergoes a bathochromic shift to 365 nm in the presence of sodium acetate, indicating a free hydroxyl group at C-7. This shift can be reversed by the addition of boric acid, indicating that there are no vicinal hydroxyl groups present.

TLC on cellulose with the solvent system isopropanol/conc. NH_3 (2:1) gave a partial separation of I and II (Table 1) and the R_f values were identical to those of authentic reference samples. 20 μg of the coumestane mixture were methylated with CH_3I and K_2CO_3. TLC on silica gel with the solvent system ether/petrol. ether (7:3) gave a partial separation into two spots which corresponded in R_f values and the colour of fluorescence to medicagol-7-*O*-methyl ether ($R_f = 0{\cdot}48$) and coumestrol-dimethyl ether ($R_f = 0{\cdot}43$).

TABLE 1. R_f VALUES OF COUMESTANES (TCL)

Compound	System I	System II	System III
Coumestrol	0·07	0·25	0·56
Trifoliol	0·31	0·27	0·66
7-Hydroxy-11,12-dimethoxycoumestone	0·22	0·15	0·54
12-*O*-Methylcoumestrol	0·30	0·33	0·96
Medicagol	0·26	0·33	0·93
Cicer-coumestanes	0·27	0·32	0·96 0·93

Key: I = silica gel, benzene/isopropanol/methanol (95:5:1). II = cellulose, 50% acetic acid. III = cellulose, isopropanol/conc. NH_3 (2:1).

The coumestane mixture was treated with 86% H_2SO_4 at room temperature. TLC on cellulose with 50% acetic acid of the ether extract of the neutralized hydrolysis mixture gave unchanged II and a new compound which was identical in R_f value (0·11) to 7,11,12-trihydroxycoumestane.[5] The mass spectrum of the mixture of permethylated coumestanes showed four intense peaks at *m/e* 310 (A), 296 (B), 295 (C) and 281 (D). The ratio of intensities of A/C and B/D remained constant, whereas the intensity ratio A/B increased in accordance with the different volatility of the compounds when the temperature of the ion source was raised. The peaks at *m/e* 310 and 295 can be assigned to medicagol-7-*O*-methyl ether and the peaks at *m/e* 296 and 281 to coumestrol-dimethyl ether.

EXPERIMENTAL

Cicer arietinum plants were grown from a commercial variety of seeds during 4 weeks in tap water.

Isolation of Coumestanes

Fresh roots (650 g) from 4-week-old *Cicer* plants were chopped in 250 ml of water in a Waring blendor. The aqueous slurry was allowed to stand for 20 hr at 25° to allow the glycosides to be hydrolysed by the glycosidases present in the roots. The mixture was worked up for phenols according to the procedure of

[6] L. JURD, *J. Org. Chem.* **24**, 1786 (1959).

Wong *et al.*[7] The ether extract was chromatographed on a cellulose column with 5·20 and 50% acetic acid. With 20% acetic acid a light fluorescent eluate was obtained which contained the coumestanes (Table 1). The coumestanes were purified twice by preparative TLC on cellulose with 50% acetic acid (R_f=0·5). Using the extinction coefficient of coumestrol, the total amount of coumestanes isolated was calculated to be 80 μg.

Mass Spectrum

Mass spectra were taken on an Atlas CH4 instrument.

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[7] E. WONG, P. J. MORTIMER and T. A. GEISSMAN, *Phytochem.* **4**, 89 (1965).