Phytochemistry, 1972, Vol. 11, pp. 3263 to 3265. Pergamon Press. Printed in England.

α-ONOCERIN AND STEROL CONTENT OF TWELVE SPECIES OF *ONONIS*

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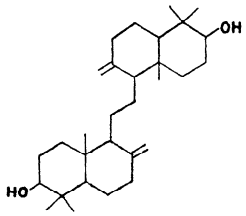
(*Received* 18 *May* 1972. *Accepted* 7 *June* 1972)

Key Word Index—*Ononis*; Leguminosae; α-onocerin; phytosterols; chemotaxonomy.

Abstract—The sterols and triterpenoids of 12 species of the genus *Ononis* were analysed by GLC. α-Onocerin was found in all but one of these species, although in some others its concentration was low. In all species examined, sitosterol was the major sterol; stigmasterol, campesterol, cholesterol and the triterpenoids cycloartenol and 24-methylene cycloartanol also occurred. The patterns of α-onocerin and sterols found seem to be consistent with the accepted classification of species within the genus.

INTRODUCTION

α-ONOCERIN (I) is a triterpenoid of unusual structure[1] which has been found in *Ononis spinosa*,[2] *O. arvensis*[3] and two species of *Lycopodium*.[4–6] We have examined the roots and aerial parts of 12 species of *Ononis* for this compound and its isomer, β-onocerin. In addition we have analysed the sterol contents of these species.



(I) α - Onocerin

Ononis belongs to the tribe Trifoleae of the Leguminosae; the currently accepted classification within the genus (containing about 70 species) is that of Sirjaev.[7] Ivimey-Cook[8] has used this classification to examine a number of taxonomic methods. Although there is no known relationship between morphological features and sterol and α-onocerin content, these may well have taxonomic value.

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[7] G. SIRJAEV, *Beih. Bot. Centr.* **49**, 381 (1932).
[8] R. B. IVEMEY- COOK, *Watsonia* **7**, 1 (1968).

RESULTS AND DISCUSSION

The species examined are shown in Table 1, as are the concentrations of α-onocerin and total 'sterols' (including cycloartenol and 24-methylenecycloartenol) in root and aerial tissues. Table 1 shows the percentages of individual sterols and triterpenoids in the 'sterol' fraction, the tentative identifications being based on GLC (1% SE30 and 1% QF1) and GLC–MS data.

α-Onocerin was found in all the species examined except *O. pusilla*. *O. viscosa* was exceptional in that it was the only species to have more α-onocerin in the aerial parts than in the roots. *Ononis cristata* and *O. pusilla* were richer in sterols than the other species. Sitosterol was the major sterol in all the species; stigmasterol, campesterol, cholesterol and the triterpenoids cycloartanol and 24-methylenecycloartenol also occurred. In general the sitosterol:stigmasterol ratio in root is lower than in aerial tissues. However, some species contain an additional sterol, probably α-spinasterol, in the root. The tendency for the sitosterol:stigmasterol ratio to be lower in roots than in aerial parts has been reported previously for maize seedlings.[9]

TABLE 1. α-ONOCERIN AND STEROL CONTENT OF *Ononis* SPECIES

Species		α-Onocerin (mg/g dry wt)	Total sterols (mg/g dry wt)	% composition of sterol fractions as determined by GLC *Rr* of peaks on 1% SE30 (relative to cholestanol)								
				1·38[a]	1·46	1·76[b]	1·92[c]	2·09[d]	2·20[e]	2·49[f]	2·76[g]	Others
O. cristata Miller[h]	*R*	5·7	3·2	2	2	12	16		55	6	7	0
	A	0·2	2·2	2	12	3	11		49	11	5	7
O. reclinata L.[i]	*R*	0	1·0	4	—	10	24		53	3	3	3
O. pubescens L.[i]	*R*	0·5	1·5	8	8	8	20		48	4	4	0
	A	0·04	2·2	3	2	5	8		74	4	3	1
O. viscosa L.[j]	*R*	0	1·4	17	8	3	22		36	6	8	0
	A	0·03	1·5	9	0	6	16		63	0	5	0
O. pusilla L[k]	*R*	0	2·8	6	—	12	20		55	3	5	0
	A	0	6·1	3	—	9	12		65	6	3	3
O. minutissima L.[l]	*R*	0·1	1·8	5	0	5	19	4	48	9	6	4
	A	0	1·2	4	2	6	9	0	68	3	4	4
O. arvensis L.[m]	*R*	5·4	1·7	2	0	9	22	16	44	4	3	0
	A	0·25	1·7	1	1	11	20	0	58	5	4	1
O. spinosa L.[n]	*R*	4·1	1·0	2	—	6	15	16	49	8	3	0
	A	0·5	1·5	8	—	9	22	0	46	2	1	3
O. repens L.[o]	*R*	2·3	1·0	1	—	7	16	5	60	3	5	4
	A	0·03	1·0	1	—	8	17	0	63	4	4	4
O. subspicata Lag.[i]	*R*	0·4	2·5	11	0	8	21		54	0	5	2
	A	0	1·5	0	4	12	16		62	3	3	0
O. mittisima L.[p]	*R*	1·2	1·7	6	8	7	23		47	3	4	2
	A	0·02	1·3	3	4	8	22		59	2	1	1
O. alopecuroides L.[q]	*R*	2·8	2·2	2	—	15	17		60	0	6	0
	A	trace	1·0	0	—	9	14		76	0	0	2

Key. a—Cholesterol; *b*—campesterol; *c*—stigmasterol; *d*—α-spinasterol; *e*—sitosterol; *f*—cycloartenol; *g*—24-methylenecycloartanol; *R*—root; *A*—aerial. Seed sources. Botanic Gardens, University of Lucerne—*h*; Botanic Gardens, University of Coimbra—*i*; Museum of Natural History, Paris—*j*; Botanic Gardens, Geneva—*k*; Botanic Gardens, Rouen—*l*; Botanic Gardens, University of Kiel—*m*; Botanic Gardens, University of Frankfurt—*n*; Botanic Gardens, Bremen—*o*; Botanic Gardens, University of Barcelona—*p*; Botanic Gardens, University of Copenhagen—*q*.

The data obtained tend to support the existing classification within the genus. Thus the three species *O. arvensis*, *O. spinosa* and *O. repens* which Sirjaev places in the same series (Vulgares) show many similarities in α-onocerin and sterol content. A particular feature is the occurrence of α-spinasterol in the roots of these three species. Two other related species which belong to subsection Bugranoides, *O. pusilla* and *O. minutissima*, have similar α-onocerin contents but *O. pusilla* is very much richer in sterols. Although both have a similar percentage composition of sterols, *O. minutissima* differs in having α-spinasterol in the roots. *Ononis pubescens* and *O. viscosa* (which Sirjaev places in the same subsection, Viscosae)

[9] R. J. KEMP, L. J. GOAD and E. I. MERCER, *Phytochem.* **6**, 1609 (1967).

also show marked similarities in their sterol content. Both have a markedly higher sitosterol : stimgasterol ratios in the aerial parts than in the roots and both contain a significant amount of the unknown component with R_t 1·46. No β-onocerin was found in any tissue.

EXPERIMENTAL

Plants. Seeds of the 12 species of *Ononis* were obtained from the sources listed in Table 1. They were grown to maturity at the University of Liverpool Botanic Gardens, and sampled at the onset of flowering.

Isolation of triterpenoids and sterols. The tissue was ground under liquid nitrogen and saponified directly. The crude non-saponifiable lipid was separated by preparative TLC on grooved chromatography plates coated with silica gel G. The plates were developed twice in 2% (v/v) MeOH in $CHCl_3$ and bands corresponding to sterol (R_f, 0·5–0·9) and α-onocerin (R_f 0·1–0·5) markers were eluted separately. Each fraction was acetylated and the acetates examined by GLC. The sterols were identified by comparison of their R_ts with those of authentic standards. Quantitative estimation of the acetates was carried out by a peak area ratio method using cholestanol as the internal standard. In a preliminary experiment this procedure was found to give more than 96% recovery of sterols and α-onocerin.

GLC. This was carried out using a Pye 104 chromatograph. A 152-cm glass column containing 1% (w/w) SE30 on Gas Chrom Q at 245° was used. 1% (w/w) QF1 at 250° was used to check the qualitative identification of the sterols. In addition the identity of some of the peaks was corroborated by combined GLC–MS using an AE1 MS12 mass spectrometer.

Acknowledgements—We would like to thank Mr. J. K. Hulme of the University of Liverpool Botanic Gardens for acquiring and growing the plants used in this study and Mrs. A. Holcroft for the determination of MS. When this work was carried out one of us (MGR) was supported by a Science Research Council Research Studentship.