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SCREENING SEED OF *TRIGONELLA* AND THREE RELATED GENERA FOR DIOSGENIN

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Abstract—Seed of 45 species of Leguminosae were analyzed for diosgenin by GLC Since the method did not separate yamogenin and tigogenin from diosgenin, results indicate maximum limits rather than the actual amounts of diosgenin present Among 27 species of *Trigonella* tested, only 6 have as much as 0 2% diosgenin, the highest was 0·8% in 1 sample of *T foenum-graecum* Among 7 species of *Medicago*, 6 species of *Melilotus* and 5 species of *Trifolium*, none contained more than 0 1% diosgenin

INTRODUCTION

THE PAPER by Fazli and Hardman[1] reporting diosgenin in *Trigonella foenum-graecum* led us to examine those species of *Trigonella* represented by seed collected for our general screening of the plant kingdom.[2] We also analyzed seed from 18 species in 3 closely related genera of the Leguminosae. Hardman *et al* [1,3–6] recorded the occurrence of other sapogenins with diosgenin in seed of 4 species of *Trigonella* and the absence of diosgenin from 6 species.

RESULTS

Of the 27 species of *Trigonella* we examined, none was richer than *T. foenum-graecum* in the component calculated as diosgenin but presumably also containing yamogenin and tigogenin.[1] Those species containing at least 0·2% diosgenin are listed in Table 1 along with the major components emerging from the gas chromatograph near diosgenin. Hardman and Fazli[4] found diosgenin in three of these species, *T. caerulea*, *T. corniculata* and *T. foenum-graecum*, and gave TLC evidence for the presence of related materials. They also reported diosgenin in *T. cretica*; our analysis of *T. cretica* showed less than 0·2% and little unidentified material. Our results by GLC are distinctly lower than those of Hardman *et al.* determined by IR spectrometry, but if the unknown components revealed by GLC are sapogenins absorbing at 900–920 cm^{-1}, the two methods could be in fair agreement.

* Agricultural Research Service, U S. Department of Agriculture

[1] FAZLI, F R Y. and HARDMAN, R. (1968) *Tropical Sci* **10,** 66

[2] BARCLAY, A S and EARLE, F R *Econ Bot* In press.

[3] HARDMAN, R and FAZLI, F R Y (1971) *Phytochemistry* **10,** 2497

[4] HARDMAN, R and FAZLI, F R Y (1972) *Planta Med* **21,** 131

[5] HARDMAN, R and JEFFERIES, T M. (1972) *Analyst* **97,** 437

[6] JEFFERIES, T M and HARDMAN, R (1972) *Planta Med* **22,** 78

Because no species was found to contain larger amounts of diosgenin than *T. foenum-graecum*, no effort was made to identify the unknown components

TABLE 1 DIOSGENIN AND SUBSTANCES WITH SIMILAR GLC BEHAVIOR IN SEED OF *Trigonella* SPECIES

Species	RR_t (tricaprin = 1)* 0·70 (%)	0·74 (%)	0·82 ± 0·02† (%)	0·84 (%)	0·86 (%)	0·94 (%)
T. caerulea (L.) Ser	0·3	Trace	0·3	0·2	Trace	Trace
T. corniculata (L.) L‡	0·1–0·2	Trace	0·1–0·3	0·1–0·2	0·1	0·1–0·2
T. fischeriana Ser	0·2	Trace	0·2	0·2	0·1	0·1
T. foenum-graecum L§	0·3–0·5	0–0·1	0·5–0·8	0–0·2	Trace	Trace
T. gladiata Stev	0·4	Trace	0·5	0·1	Trace	Trace
T. sibthorpii Boiss	0·2	0·1	0·2	0·1	Trace	Trace

* Relative retention times of other components vary in a pattern similar to that of diosgenin. Calculation assumes all components have the same detector response as diosgenin.

† Diosgenin. Probably includes yamogenin, tigogenin and perhaps other components.

‡ Two samples.

§ Six samples.

The following *Trigonella* species contained less than 0·2% diosgenin, usually less than 0·1% and, in general, less of the unknown materials than those species listed in Table 1: *T. anguina* Del., *T. arabica* Del., *T. arcuata* C. A. Mey., *T. balansae* Boiss. & Reut., *T. brachycarpa* (Fisch.) Moris, *T. caelesyriaca* Boiss., *T. calliceras* Fisch., *T. cretica* (L.) Boiss., *T. emodi* Benth., *T. incisa* Benth., *T. kotschyi* Fenzl ex Boiss., *T. monantha* C. A. Mey., *T. monspeliaca* L., *T. noaeana* Boiss., *T. orthoceras* Kar. & Kir., *T. polycerata* L., *T. rigida* Boiss. & Bal., *T. spicata* Sibth. & Sm., *T. stellata* Forsk., *T. suavissima* Lindl., *T. uncata* Boiss. & Noe.

None of the other species tested (listed below) had more than 0·1% diosgenin, but *Medicago* differed from *Melilotus* and *Trifolium* by having 0·1–0·6% of a component with relative retention time (RR_t) of 0·60, whereas the other two genera had none. *Medicago arabica* (L.) Huds., *M. ciliaris* (L.) All., *M. polymorpha* L., *M. orbicularis* (L.) Bartal., *M. radiata* L., *M. sativa* L., *M. turbinata* (L.) All., *Melilotus alba* Desr., *M. indica* (L.) All., *M. messanensis* (L.) All., *M. neapolitana* Ten., *M. officinalis* (L.) Pallas, *M. sulcata* Desf., *Trifolium amtabense* Boiss. & Hausskn., *T. campestre* Schreb., *T. fragiferum* L., *T. hybridum* L., *T. nigrescens* ssp. *petrisavii* (Clem.) Holmboe

EXPERIMENTAL

Sapogenins were concentrated by essentially the same procedure used by Hardman *et al.*[3,6] the residue from acid hydrolysis 1·5 g seed was dried overnight at room temp. rather than in an oven, and the sapogenins were extracted with petrol in a Butt apparatus for 6 hr instead of in a Soxhlet for 24 hr. To measure sapogenins we used a gas chromatograph with a 2·5 ft × 1/8 in. stainless-steel column packed with 3%, OV-1 on Gas-Chrom Q. Temp. was programmed from 150° at 4°/min, and the R_t of diosgenin relative to tricaprin varied from 0·80 to 0·84. Tigogenin was not separated from diosgenin. RR_t on SE-30 for yamogenin is so near those for diosgenin and tigogenin[7] that presumably it too would not be separated from diosgenin on OV-1, yamogenin was not available for testing.

The presence of other components in the diosgenin peak is not ruled out. When 1·6% and 1·9% diosgenin was added to two portions of ground soybeans [*Glycine max* (L.) Merr.] essentially free of diosgenin (< 0·02%), analysis indicated 1·8% and 1·9%, respectively. After addition of 0·5% and 2·3% to portions of *Trigonella foenum-graecum* containing 0·6% of native diosgenin, analysis showed 1·0%, and 2·6%. Recovery was considered adequate for our survey.

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[7] VANDEN HEUVEL, W. J. A. and HORNING, E. C. (1961) *J. Org. Chem.* **26**, 634