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STEROLS AND FATTY ACIDS OF SOME NON-PHOTOSYNTHETIC ANGIOSPERMS*

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Key Word Index—*Cuscuta campestris*; Convolvulaceae; *Monotropa uniflora*; *M. hypopitys*; Pyrolaceae; fatty acids; sterols; non-photosynthetic angiosperms.

Abstract—Sterols and fatty acids were extracted and identified from three parasitic angiosperms, *Cuscuta campestris*, *Monotropa uniflora* and *M. hypopitys*. Each plant contained the typical 16 and 18-carbon fatty acids of angiosperms, but the partially-photosynthetic *Cuscuta* contained much larger quantities of linolenic acid than the non-green *Monotropa* species which had smaller amounts of linolenic acid characteristic of non-photosynthetic tissue. Sterol quantity was three times higher in *Cuscuta* than in the *Monotropa* species. Sitosterol was the major sterol in all species with smaller amounts of campesterol and cholesterol.

INTRODUCTION

While the isolation and identification of sterols and fatty acids in photosynthetic higher plants has been under extensive study for the past decade, research on the fatty acids and sterols of non-photosynthetic higher plants has been almost completely ignored. One report, by Rhomer *et al.* [1], determined that the sterols of two non-photosynthetic higher plants, *Cuscuta epithymum* and *Orobanche lutea*, were similar to the sterols of photosynthetic higher plants. The purpose of this study was to extract and identify the fatty acids and sterols of three non-photosynthetic seed plants common to eastern North America.

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RESULTS AND DISCUSSION

The major fatty acid from *Cuscuta campestris* was linolenic acid, followed by linoleic acid and palmitic acid. Small amounts of stearic acid and oleic acid were also identified from the sample (Table 1).

Analyses of the sterols of *C. campestris* showed sitosterol as the major sterol, followed by campesterol and stigmasterol. A rather high concentration of cholesterol was identified in an amount similar to that of campesterol. In *C. epithymum*, cholesterol was found only in trace amounts [1].

Except for the presence of small amounts of hexadecenoic acid (16:1), the fatty acids of *Monotropa uniflora* were qualitatively similar to those of *Cuscuta*. However, the quantities of the individual fatty acids differed considerably. The major fatty acid for *M. uniflora* was linoleic acid, followed by palmitic acid.

Table 1. Fatty acid and sterol composition of three non-photosynthetic angiosperms*

Fatty acids	*Cuscuta campestris*	*Monotropa hypopitys*	*Monotropa uniflora*
16:0	25	26	23
16:1	—	—	3
18:0	3	2	4
18:1	2	11	9
18:2	29	55	49
18:3	41	6	11
Sterols			
cholesterol	12	1	1
campesterol	11	11	12
stigmasterol	6	—	—
sitosterol	72	79	87
cycloartenol		5	
cycloeucalenol		4	
Total fatty acids (% dry weight)	0.19	0.19	0.22
Total sterol (% dry weight)	0.18	0.05	0.04

*Fatty acid data expressed as % of total fatty acid; sterol data expressed as % of total sterol.

The major sterol of *M. uniflora* was sitosterol, campesterol ranked second, with trace amounts of cholesterol. Stigmasterol was not detected.

The lipids extracted from *M. hypopitys* were similar both qualitatively and quantitatively to *M. uniflora*, except for the absence of the fatty acid 16:1 (Table 1). The sterol composition was similar to that of *M. uniflora*, except for two minor unknowns which were tentatively identified as cycloartenol and cycloeucalenol. However, insufficient material was available for a detailed characterization.

Comparison of the lipids of all three non-photosynthetic higher plants show that the individual fatty acids of *C. campestris* differ quantitatively from the fatty acids of *Monotropa uniflora* and *M. hypopitys* especially with respect to linolenic acid composition. Triunsaturated fatty acids are characteristic of photosynthetic eucaryotes and it has been suggested that α-linolenic acid is involved in photosynthetic oxygen evolution [2]. In the early stages of its life cycle *Cuscuta* contains chlorophyll. The linolenic acid composition of *Cuscuta* may reflect the presence of chloroplasts in the organism or the fact that it was grown as a parasite on green tissue (in this case, buckwheat). Both *Monotropa* species have low linolenic acid composition characteristic of non-green tissue.

Comparison of the sterols, show that *M. hypopitys* and *M. uniflora* are almost identical in their composition except for the two unknowns tentatively identified as cycloartenol and cycloeucalenol in *M. hypopitys*. Rhomer *et al.* [1] also identified these sterols in their work with *Cuscuta epithymum* and *Orobanche lutea*. It has been suggested that the presence of cycloartenol is closely connected with the presence of a photosynthetic apparatus in the tissue [1, 3]. Since photosynthetic eucaryotes are characterized by a sterol biosynthetic pathway involving cycloartenol, as opposed to lanosterol in non-photosynthetic eucaryotes, the presence of cycloartenol and its derivatives indicates a typical higher plant pathway [3]. Therefore, the absence of chloroplasts in higher plants or in dark grown *Euglena* [4] does not appear to modify the cycloartenol biosynthetic pathway for sterols.

The total sterol content for both *M. hypopitys* and *M. uniflora* is approximately one-third less than reported for most photosynthetic higher plants [5, 6]. This could be accounted for by the total lack of chloroplasts in *Monotropa* as compared to *Cuscuta campestris* which is partially photosynthetic [2].

Analyses of the results for all three parasitic angiosperms show only sterols common to higher photosynthetic plants. The fatty acids present in the three angiosperms studied were within the range of values which would be expected for photosynthetic angiosperms although the linolenic acid composition of the two *Monotropa* species is more typical of non-green tissue [5, 6]. Therefore, the collective data in this study suggests that the fatty acids and sterols of non-photosynthetic angiosperms are both qualitatively and quantitatively similar to the fatty acids and sterols of photosynthetic rather than non-photosynthetic eucaryotes.

EXPERIMENTAL

Monotropa uniflora (indian pipe) and *Monotropa hypopitys* (pinesap) were collected in southern and central Maryland. *Cuscuta campestris* (dodder) was grown in the greenhouse. Each sample was freeze-dried and ground in a Wiley mill before extraction.

Extraction of fatty acids and sterols. Lipids were extracted from the dried tissue with $CHCl_3$–MeOH (2:1) in a Soxhlet for 24 hr. The solvent was evapd and the extract redissolved in $CHCl_3$ and filtered to remove particulate matter. The total lipid remaining after evapn of $CHCl_3$ was saponified with a 20% soln of KOH (2 × weight of lipid) in 70% EtOH by refluxing for 1 hr. The soln was cooled and acidified with 6M HCl to pH 3. The lipids were extracted with Et_2O in a liquid–liquid extractor for 12 hr. The Et_2O fraction was collected and evapd. The residue was dissolved in BF_3–MeOH and heated for 10 min to esterify fatty acids. The soln was partitioned 5 : against 20 ml hexane. The hexane fractions were combined and placed on a Woelm Grade II Al_2O_3 column and eluted with hexane, hexane–C_6H_6 (1:1) (fatty acid esters), C_6H_6 (fatty acid esters), Et_2O (sterols), and Et_2O–MeOH (9:1) (sterols).

Identification of fatty acids and sterols. Fatty acid esters were analyzed by GLC on a 15% HI-EFF 1BP column at 160°. TLC on $AgNO_3$–Si gel was also used for fatty acid ester identification. The sterols were analyzed by GLC on a 3% SE-30 column at 245°. Identifications were made on the basis of retention times relative to free cholesterol and confirmed by GC–MS [7].

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