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MINOR PHOSPHOLIPIDS OF THE KERNELS OF THE SEEDS OF THE COTTON PLANT OF VARIETY 159-F

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We have previously reported [1, 2] that in the cotton plant at various stages of development a phosphatidic acid (PA) has been detected which has been identified by qualitative reactions and chromatographic mobility and on the basis of spectrometric information. It must be mentioned that contradictory information is given in the literature on the chromatographic mobilities of the phosphatidic acids. Some authors give high values of R_f in an ammonia system of silica gel — 0.75 [3], 0.68 [4], 0.8 [5]; others indicate a low mobility under the same conditions — 0.04 [6], 0.07 [7], 0.05 [8]. For a more rigid identification of the phosphatidic acid isolated we have obtained the corresponding compound from phosphatidylcholine by enzymatic hydrolysis with phospholipase D [8]. The phosphatidic acid obtained was compared with that isolated from the cotton plant and their complete identity was established. R_f values of phosphatidic acids given in the second set of publications mentioned [6-8] have been fully confirmed.

We also isolated a phosphatidic acid from ripe cotton seeds. The amount of phosphatidic acid in the total phospholipids was 0.3% in terms of phosphorus, and its fatty acid composition was: 16:0 — 50.2%; 18:0 — traces; 18:1 — 24.4%; 18:2 — 25.4%; 18:3 — traces.

With the aid of one-dimensional and two-dimensional TLC on silica gel in systems 1 and 2 [1] a phosphorus-containing compound with R_f 0.15 (system 1) and 0.12 (system 2) was detected in, and was then isolated from, the total phospholipids of cottonseed kernels, its proportion amounting to 0.1% in terms of phosphorus of the total phospholipids and its fatty acid composition being: 14:0 — traces; 16:0 — 58.8%; 18:0 — traces; 18:1 — 19.8%; 18:2 — 21.4%. From the results of chromatographic mobility, qualitative reactions, IR spectra, products of acid hydrolysis, information in the literature [6], and comparison with lysophosphatidylinositol obtained by enzymatic hydrolysis from phosphatidylinositol by phospholipase A_2, we established that this phospholipid is identical with lysophosphatidylinositol.

To confirm the presence of phosphatidic acid and lysophosphatidylinositol in the cottonseed kernels we isolated the total phospholipids from samples of cotton seeds harvested in different years. We detected phosphatidic acid and lysophosphatidylinositol in all the combined phospholipids from which it follows that these phospholipids are component parts of the biological membranes and not artifacts.

We have detected phosphatidic acid and phosphophatidylinositol among the phospholipids of cottonseed kernels from variety 159-F for the first time.

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COUMARINS OF *Ferula conocaula*

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The isolation of a number of terpenoid coumarins from the roots of *Ferula conocaula* Korov. has been reported [1, 2]. In a further study of the plant, from ethanolic and aqueous ethanolic extracts by chromatography on a column of silica gel we have isolated another five compounds of coumarin nature: (I), $C_{24}H_{30}O_3$, mp 61-63°C; (II), $C_{24}H_{30}O_4$, mp 114-116°C; (III), $C_9H_6O_3$, mp 230-232°C; (IV), $C_{26}H_{32}O_5$, mp 160-162°C; and (V), $C_{36}H_{50}O_{15}$, mp 161-162°C, $[\alpha]_D^{25}$ −90°C (c 1.0; ethanol).

From their physicochemical constants and spectral (IR, PMR) characteristics, substances (I-IV) were identified as umbelliprenin, fesolol, and feterin, respectively.

According to its UV spectrum, the new compound (V), which has been called cauloside, is an umbelliferone derivative. The low R_f value of the substance and its UV and IR spectra show that it is a glycosylated terpenoid coumarin.

On acid hydrolysis, the glycoside gave umbelliferone and D-glucose. The acetylation of (V) with acetic anhydride in pyridine led to an octaacetate, $C_{52}H_{66}O_{23}$, M^+ 1058. Consequently, the glycoside is a bioside.

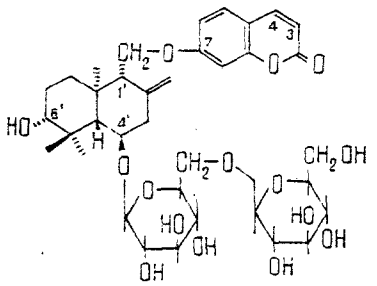
Enzymatic cleavage of the glycoside with β-glucosidase [3] yielded D-glucose and an aglycone with the composition $C_{24}H_{30}O_5$, M^+ 398. The latter, from a comparison of its PMR and IR spectra and also its physicochemical constants, was found to be identical with cauferin [1].

Since there are two secondary hydroxy groups in cauferin, the position of the sugar residue was established by comparing the PMR spectra of the aglycone, of the glycoside, and of its octaacetate. In the PMR spectrum of the octaacetate, as compared with the glycoside and the aglycone, the quartet signal of the hemihydroxylic proton at C_6' shifted downfield. Consequently, the hydroxy group in the glycoside at C_6' is free and the sugar residue consists of two glucose molecules and is located at C_4'.

The bond between the carbohydrates was established by the Hakomori methylation of (V) followed by the acid hydrolysis of the permethylate obtained. In the hydrolysate the methylated carbohydrates were identified on the basis of a combination of GLC and TLC methods as 2,3,4-tri-O-methyl-D-glucose and 2,3,4,6-tetra-O-methyl-D-glucose. Consequently, the sugars are linked by a (1 → 6) bond.

The presence in the IR spectrum of the glycoside of absorption bands at 1100, 1081, 1038, and 889 cm^{-1} shows that the glucose residues have the pyranose form and are linked by a β-glycosidic bond, as was confirmed by enzymatic hydrolysis [4-6].

On the basis of the facts presented above, it may be assumed that the glycoside is cauferin 4'-O-(O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside).



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