Phytochemistry, 1970, Vol. 9, pp. 667 to 669. Pergamon Press. Printed in England.

SHORT COMMUNICATION

ISOFERULIC ACID CHOLINE ESTER: A NEW PLANT CONSTITUENT

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(*Received* 15 *September* 1969)

Abstract—A fluorescent cation in seed extracts of the crucifer *Sibara virginica* (L). Rollins has been isolated, combined with thiocyanate ion and identified as (I), the choline ester of isoferulic acid (3-hydroxy-4-methoxy-cinnamic acid). The new compound is structurally similar to sinapin (II), the well-known choline ester of sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid).

INTRODUCTION

IN THE course of studies aimed at clarifying the glucosinolate distribution in seed extracts of the crucifer *Sibara virginica* (L). Rollins and reported elsewhere,[1] the discovery of a strongly fluorescent component, noted on chromatography, attracted our interest. Similar fluorescent constituents have been repeatedly observed in extracts of crucifer seeds, but have mostly been attributed to sinapin (II), a well-known ester of choline and sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid) which is widely distributed in the Cruciferae.[2] We now report the isolation from *S. virginica* seeds of a new, structurally similar ester of choline and isoferulic (hesperetic) acid (I), responsible for the observed fluorescence on the chromatograms.

RESULTS AND DISCUSSION

Seeds of *Sibara virginica* (L). Rollins, a crucifer native in the Southern United States, were collected on the campus of Rice University, Houston, Texas, by Dr. M. G. Ettlinger and kindly placed at our disposal. A larger seed sample was subsequently produced by cultivation of the species in the Botanic Garden of the University of Copenhagen.

Paper chromatograms of methanolic seed extract of *S. virginica* invariably exhibited a spot with almost the same R_f as sinapin, but differing from the latter by a less brilliant, blue fluorescence in u.v. light and by not turning yellow on exposure to ammonia vapour. With diazotized sulphanilic acid, the unknown compound produced a brick-red colour.

A methanolic seed extract of *S. virginica*, serving as the source of glucosinolates, was processed as described in another paper.[1] After enzymatic hydrolysis and chloroform extraction of the resulting products (isothiocyanates and a ring-closed derivative), the aqueous phase was concentrated to a small volume. Addition of thiocyanate ions caused separation of a crystalline salt. Alternatively, the glucosinolates from 150 g of seeds were removed from the extract by means of a Dowex 1 × 1 anion exchange resin; the eluate was concentrated to a small volume and ammonium thiocyanate was added, resulting in the separation of a salt

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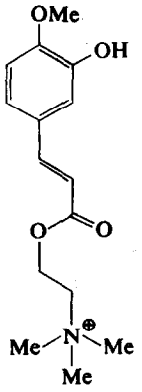
[1] R. GMELIN, A. KJÆR and A. SCHUSTER, *Acta. Chem. Scand.* **24**, (1970) in press.

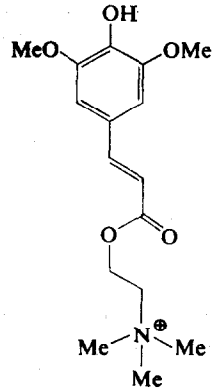
[2] R. HEGNAUER, *Chemotaxonomie der Pflanzen*, Vol. III, p. 594, Birkhäuser Verlag, Basel and Stuttgart (1964).

(670 mg) which, after recrystallization from water, possessed the elementary composition $C_{16}H_{22}N_2O_4S$. The cation, $C_{15}H_{22}NO_4$, displaying ester carbonyl absorption in its i.r. spectrum, was hydrolysed by hot alkali to give an acid and a nitrogen-containing alcohol. The latter was identified as choline by production of the crystalline dipicrylamine salt.[3] The acid, $C_{10}H_{10}O_4$, was easily identified as isoferulic acid on the basis of its spectroscopic properties (u.v., i.r., NMR, and mass) and, eventually, by comparison with an authentic specimen.

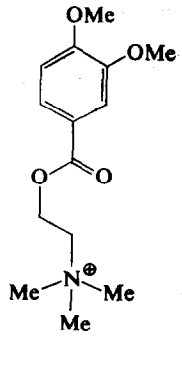
Consequently, the fluorescence constituent in *S. virginica* is the choline ester of isoferulic acid (I), a new compound formally resembling sinapin (II), a common seed constituent in the Cruciferae.[2] Another structurally related ester, yet derived from a benzoic acid, is hesperalin (III), identified a few years ago as a constituent of seed of the crucifer *Hesperis matronalis* L.[3] The function and biological synthesis of these choline esters are unknown.



(I)



(II)



(III)

Isoferulic acid, the acid moiety of (I), is not widely distributed in nature. Reports are available on its presence in rhizomes of *Cimicifuga racemosa* (Ranunculaceae),[4] root bark of *Catalpa ovata* (Bignoniaceae)[5] and rhizomes and roots of *Valeriana officinalis* (Valerianaceae).[6] In addition, its methyl ester has been reported as a constituent of the fungus *Lentinus lepideus* (Basidiomycetes),[7] probably biosynthesized in this species from phenylalanine rather than tyrosine.[8]

EXPERIMENTAL

M.p.s are uncorrected and determined in capillary tubes in an electrically heated bath.

Isolation of Isoferulic Acid Choline Ester

(i) Ground, defatted seeds of *Sibara virginica* (85 g; from 100 g of fresh seeds) were extracted with two 600-ml portions of 70% methanol. The combined filtrates were concentrated and a 20% lead acetate solution was added to precipitate impurities. After filtration, an excess of 20% Na_2HPO_4 solution was added, and the salt removed by filtration. The filtrate was diluted to 1 l. with a citrate buffer (pH 6·4); a myrosinase solution

[3] R. GMELIN and H. MÖHRLE, *Arch. Pharm.* **300**, 176 (1967).
[4] H. FINNEMORE, *Pharm. J.* **83**, 145 (1909).
[5] M. HIRAMOTO and K. WATANABE, *J. Pharm. Soc.* **59**, 261 (1939).
[6] A. STOLL and E. SEEBECK, *Ann.* **603**, 158 (1957).
[7] H. SHIMAZONO, *Arch. Biochem. Biophys.* **83**, 206 (1959).
[8] D. M. POWER, G. H. N. TOWERS and A. C. NEISH, *Can. J. Biochem.* **43**, 1397 (1965).

(5 ml) and a trace of ascorbic acid were added and the mixture was set aside for 4 hr at ambient temperature. The isothiocyanates and 2-thiooxazolidone, produced in the enzymatic reaction,[1] were extracted with $CHCl_3$. The aqueous phase was concentrated to 50 ml *in vacuo*, and NH_4SCN (1 g) was added. After several days in the refrigerator, crystals separated. They were filtered off and recrystallized several times from water to give 140 mg of the pure thiocyanate (*vide infra*).

(ii) Alternatively, the following isolation procedure was employed: seeds of *S. virginica* (150 g) were ground, defatted and extracted with 70% methanol as described above. The extracts were concentrated *in vacuo* to a small volume, water was added to a total volume of 1 l. and the solution was filtered through celite. The filtrate was slowly passed through a column (50 cm × 2 cm) of Dowex 1 × 1 anion exchange resin in the chloride form. The column was rinsed with water (250 ml). The filtrate, combined with the washings, was concentrated to about 25 ml in a vacuum, and NH_4SCN (1 g) was added to the solution. Crystals (667 mg) separated and were collected after 2 days in the refrigerator. An analytical sample (487 mg) was produced by recrystallization from water, with the addition of norite. M.p. 152–153°, $\lambda_{max}^{H_2O}$ 218 nm (ε 14,200), 230 nm (infl., ε 10,000), 300 nm (shoulder, ε 13,200), and 325 nm (ε 16,000), displaced in alkali to 227 nm (ε 18,000), 263 nm (ε 20,700), 305 nm (ε 15,200), and 360 nm (ε 12,900). The i.r. spectrum (KBr) exhibited strong bands at 2030 (SCN^-) and 1700 cm^{-1} (ester). (Found: C, 56·79; H, 6·61; N, 8·34; S, 9·45. $C_{16}H_{22}N_2O_4S$ required: C, 56·78; H, 6·55; N, 8·28; S, 9·48%).

Alkaline Hydrolysis of the Choline Ester

The choline ester (140 mg) was suspended in 10% NaOH (10 ml) and heated to boiling for 5 min. After cooling, the reaction mixture was acidified with dilute H_2SO_4, and the precipitated acid was removed by filtration and recrystallized from dilute ethanol to give colourless needles (31 mg), m.p. 234–235°, alone or in admixture with a commercial specimen of isoferulic acid. Furthermore, the acid possessed the reported u.v. spectrum in ethanol, with or without added base,[9] as well as in methanol, with or without added $AlCl_3$.[10] I.r. and mass spectra further served to confirm the identity.

To the neutralized, aqueous filtrate was added a saturated solution of ammonium dipicrylaminate, causing immediate precipitation of the red choline salt, m.p. 232–234°, alone or in admixture with an authentic specimen.[3]

Paper Chromatography of the Choline Ester

On ascending paper chromatography, with the upper phase of *n*-BuOH–EtOH–H_2O (4:1:4) as the mobile phase (Schleicher and Schüll paper No. 2043b), the choline ester migrated with an R_f of 0·40. The spot was detected in u.v. light by its pale bluish fluorescence. On exposure to ammonia, the spot did not turn yellow, contrary to that of sinapin. The R_fs of sinapin thiocyanate and hesperalin iodide,[3] chromatographed simultaneously, were 0·40 and 0·42, respectively. Like these two ester salts, the new choline ester (I) gave an orange-brown spot with Dragendorff's reagent. With PtI_6^{--}, a bluish spot was produced and with diazotized sulphanilic acid an intense brick-red colour resulted.

Acknowledgements—The authors are most grateful to Dr. M. G. Ettlinger for supplying *Sibara* seed material. Microanalyses were performed by Mr. G. Cornali and his staff. The competent technical assistance of Miss A. Schuster is appreciated. The support of the present investigation by *Statens Tekniske-Videnskabelige Fond* is gratefully acknowledged.

[9] S. El-Basyouni and G. H. N. Towers, *Can. J. Biochem.* **42**, 493 (1964).
[10] Y. Nakagawa, M. R. Shetlar and S. H. Wender, *Anal. Biochem.* **7**, 374 (1964).