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SHORT COMMUNICATION

THE ANTIFUNGAL FACTORS IN BARLEY—III.

ISOLATION OF p-COUMAROYLAGMATINE*

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Abstract—A basic compound which exhibits weak antifungal activity was isolated from young barley shoots and characterized as the picrate. It was identified as the previously unknown *p*-coumaroylagmatine and its structure confirmed by synthesis.

INTRODUCTION

THE preceding communication[1] in this series showed that the antifungal principle[2] extracted from young barley shoots is associated with a basic fraction which can be further separated into several components of varying degrees of activity. One of these, which occurs in only small amount, appeared to have only little antifungal potency. Its other properties, however, suggested that it was chemically related to the other bases but had a simpler structure. Its characterization therefore became desirable.

RESULTS

In the present work, repeated countercurrent distribution of the basic extract in the system *n*-butanol–water–acetic acid, was found more convenient than the butanol–water distribution previously employed. A fraction corresponding to Fraction VIII of the previous communication,[1] was characterized by maximal R_f value and relatively intense absorption in the u.v. It furnished a picrate, in a yield of 50 mg from 20 kg coleoptiles, which was readily purified by recrystallization.

The picrate ion absorption at 355 mμ allowed calculation of the equivalent weight of the salt as approximately 510. A further peak in the u.v. spectrum at 310 mμ was associated with an extinction of 19,000 (after correction for picrate absorption) and represented the absorption of the parent base. The hydrochloride, which was prepared in solution only, exhibited the u.v. spectrum characteristic of derivatives of *p*-coumaric acid, with $\lambda\lambda_{max}$ 304, 292, 220 mμ (ϵ 21,000, 22,000, 18,000 respectively). The high extinction near 300 mμ showed that the *trans*- rather than the *cis*-isomer[3] was present. In alkaline solution, the maximum was displaced to 342 mμ, indicating that the *p*-hydroxyl group was free.

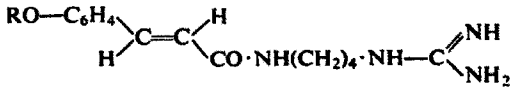
A positive Sakaguchi reaction suggested the presence of a free guanidino group, a conclusion consistent with the strongly basic character of the compound. Hydrolysis led to the

* Contribution No. 303; Refs. 3 and 4 are to be regarded as Parts II and I respectively.

[1] K. KOSHIMIZU, E. Y. SPENCER and A. STOESSL, *Can. J. Botany* **41**, 744 (1963).
[2] R. A. LUDWIG, E. Y. SPENCER and C. H. UNWIN, *Can. J. Botany* **38**, 21 (1961).
[3] J. J. BLOOMFIELD and R. FUCHS, *J. Org. Chem.* **26**, 2991 (1961).

liberation of agmatine; the coumaric acid moiety itself was apparently destroyed under the hydrolytic conditions employed.

These data suggested that the structure of the metabolite was that of *p*-coumaroylagmatine (1-[*trans*-*p*-hydroxycinnamoylamino]-4-guanidinobutane, I, R = H). Proof that this was



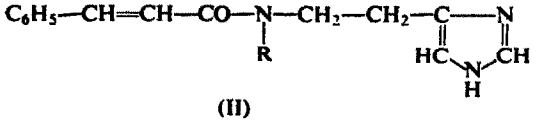
(I)

indeed the case was obtained by a synthesis of the compound, from agmatine monohydrochloride and methyl-*trans*-*p*-coumarate, which was essentially identical with the isolated metabolite but contained a trace of an impurity which could not be removed by further recrystallizations. Methylation of both the metabolic and the synthetic compounds afforded the same monomethyl ether which was isolated as the crystalline hydrochloride. This was identical in every respect with an authentic specimen of *p*-methoxycinnamoylagmatine hydrochloride (I, R = Me) prepared by the acylation of agmatine with *p*-methoxycinnamoyl chloride.

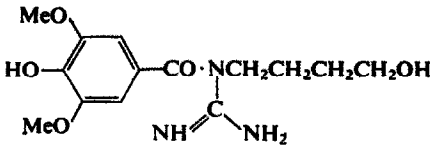
Appropriate tests showed that purified *p*-coumaroylagmatine possesses only low antifungal activity (Table 1). Its isolation is nevertheless of interest because recent work elsewhere[4] has provided evidence that the biosynthesis of putrescine by barley proceeds on a pathway involving agmatine. It should be noted that agmatine is also a moiety[5] of the major antifungal components referred to above and is therefore metabolized by barley in substantial amounts. *p*-Coumaroylagmatine itself does not appear to have been described before but some closely related, naturally occurring compounds are represented by N^{α}-cinnamoylhistamine[6] (II, R = H), its N^{α}-methyl derivative (II, R = Me) as a moiety of the alkaloid casimiroedine,[7] and leonurine[8] (III). *p*-Coumaric acid is widely distributed in vascular plants and is important in the biosynthesis of lignin; it has generally been found in the form of esters.[9]

TABLE 1. ANTIFUNGAL ACTIVITY OF *p*-COUMAROYLAGMATINE AGAINST *Monilinia fructicola*

ppm	224	112	56	28
% inhibition	100	78	24	0



(II)



(III)

[4] T. A. SMITH and J. L. GARRAWAY, *Phytochem.* 3, 23 (1964).
[5] To be published.
[6] J. S. FITZGERALD, *Austral. J. Chem.* 17, 375 (1964).
[7] S. RAMAN, J. REDDY, W. LIPSCOMB, A. L. KAPOOR and C. DJERASSI, *Tetrahedron Lett.* 1962, 357.
[8] T. GOTO, N. KATO, Y. HIRATA and Y. HAYASHI, *Tetrahedron Lett.* 1962, 545.
[9] S. Z. EL-BASYOUNI, A. C. NEISH and G. H. N. TOWERS, *Phytochem.* 3, 627 (1964); and references cited there.

EXPERIMENTAL

Unless otherwise stated, melting points were determined on the Kofler block and u.v. spectra in 96% ethanol. Evaporations were *in vacuo* below 40°. Microanalyses were by Dr. C. Daessle, Montreal.

Countercurrent Distribution of Metabolic Products

The basic fraction of the water-soluble products of young barley shoots was obtained by ion-exchange as previously described.[1] The fraction (4·5–6·5 g) was distributed by the single withdrawal procedure[10] in *n*-BuOH:H_2O:HOAc (4:5:1) (100 × 25 ml lower phase; 300 × 25 ml upper phase). Fractions $\rho = 0$ to $\rho = 199$ were collected together and evaporated to dryness *in vacuo*. The products of five such runs (from a total of 20 kg of barley shoots) were combined, the aqueous solution washed with ether and the ether-insoluble material redistributed in the same solvent system (100 × 25 ml lower phase, 169 × 25 ml upper phase). Tubes $\rho = 49$ to $\rho = 58$ contained material (30 mg, λ_{max} 290 mμ, $E^{1\%}_{1cm}$ 330) which gave rise to a single basic Sakaguchi-positive spot, R_f 0·7 (in *n*-BuOH–H_2O–HOAc 4:5:1, Whatman No. 1 paper). Tubes $\rho = 59$ to $\rho = 68$ contained similar material (35 mg) of $E^{1\%}_{1cm}$ 455.

Isolation of the Picrate

On addition of picric acid to the above materials in water (3 ml), a precipitate separated which crystallized on standing. After washing with water, the salt (50 mg) had m.p. 214°, raised to 215–217° by recrystallization from aqueous methanol and aqueous ethanol. It had $\lambda\lambda_{max}$ 355, 310, 292 (sh) and 223 mμ ($E^{1\%}_{1cm}$ 291, 493, 451, 512 respectively).

Hydrolysis of the Picrate

The salt (2·2 mg) was heated on the steambath with 2 N hydrochloric acid (0·5 ml) for 22 hr, when the solution was extracted with ether. Starting material and agmatine could be detected in the aqueous product by thin-layer chromatography (on Avicel* in *n*-BuOH: H_2O:HOAc (4:5:1)). The ether-soluble fraction contained only picric acid.

Ultraviolet Spectrum of the Parent Base

The picrate (0·31 mg) in 50% aqueous ethanol was passed through a small column of Dowex 2-X 8 (Cl^-) and the column washed with 50% aqueous ethanol to produce 25 ml of combined filtrates. The spectroscopic data of this solution are recorded in the text. Two drops of 0·1 N sodium hydroxide were added to 4 ml of solution to produce alkaline conditions.

Antifungal Activity

The picrate (2 mg) was ion-exchanged on a small column of Dowex 2-X 8 (OAc^-) as above. The filtrates were evaporated *in vacuo* and the residue taken up in water. The concentration of the solution was estimated by spectroscopy which also verified the absence of picric acid. Bioassay by the standard spore drop assay, using *Monilinia fructicola* as test organism, gave the results shown in Table 1.

* A microcrystalline cellulose from FMC Corporation, American Viscose Division, Newark, Delaware, U.S.A.

[10] For the terminology employed see E. HECKER, *Verteilungsverfahren im Laboratorium*, Verlag Chemie, Weinheim (1955).

p-*Coumaroylagmatine Picrate*

Agmatine dihydrochloride (153 mg) was evaporated *in vacuo* from 1 N sodium hydroxide solution (0·76 ml, 1 mol-equiv.). The product was heated together with methyl-*trans-p*-coumarate[11] (270 mg, 2·0 mol-equiv.) at 120° for 24 hr, taken up in water containing a little methanol and washed with several portions of ether. The aqueous fraction, on repeated preparative thin-layer chromatography (on Avicel in *n*-BuOH:H_2O:HOAc (4:5:1), ten plates, 20 × 20 cm, in the first run, eight plates in the second) furnished the desired product as a syrup (75 mg) which was precipitated from water as the picrate. After recrystallization from aqueous ethanol, the salt had m.p. 215–218° dec., also on admixture with the picrate of the natural compound (Found: C, 47·9; H, 4·7; N, 19·1. $C_{20}H_{23}N_7O_9$ required: C, 47.5; H, 4·6; N, 19·4%). Infrared spectra (Nujol mull and solution in dimethylsulphoxide) of the two preparations were identical, as were also the u.v. spectra. The chromatographic behaviour of both products, in four different systems, was identical except that a small amount of an impurity could be detected in the synthetic material.

p-*Methoxycinnamoylagmatine Hydrochloride*

p-Methoxycinnamic acid,[12] 185 mg, was refluxed with thionyl chloride (1 ml) for 10 min. The residue after evaporation was treated overnight with agmatine sulphate (230 mg) in pyridine (2 ml) at room temperature. The solid was filtered off, washed with a few drops of pyridine and extracted with methanol (8 ml, 5 ml) at room temperature. Filtration of the methanol-soluble material through Dowex 2-X 8 (Cl^-) in water afforded the desired product as the hydrochloride (96 mg). After repeated recrystallization from water, it had m.p. 212–215° and $\lambda\lambda_{max}$ 304, 290, 223 mμ (ϵ 23,000; 23,900; 14,600) (Found: C, 54·8; H, 6·8; N, 16·9; Cl, 11·1. $C_{15}H_{23}N_4O_2$ Cl required: C, 55·1; H, 7·1; N, 17·2; Cl, 10·9%).

Methylation of p-*Coumaroylagmatine*

The hydrochloride (6·5 mg) derived by ion-exchange from metabolite picrate was refluxed for 30 min. in methanol (2 ml) together with potassium carbonate (60 mg) and dimethylsulphate (0·038 ml). After dilution with water, the solution was filtered in turn through Dowex 2-X 8 (OAc^-) and Rexyn CG 51 (H^+). The latter column was washed with water and eluted with 2 N acetic acid (75 ml). Preparative chromatography (on Avicel, three plates, as above) of the eluate gave the desired methyl ether (located by u.v. absorption). After evaporation from a few drops of 0·1N hydrochloric acid, it crystallized from methanol/acetone. Recrystallized from water, it had m.p. 207–210°, and m.p. 209–213° on admixture with the authentic *p*-methoxycinnamoylagmatine hydrochloride. The u.v. (unchanged by addition of OH^-) and i.r. (KBr disc) spectra of the two specimens were, respectively, identical.

Similar methylation of synthetic *p*-coumaroylagmatine gave the same compound, again characterized by m.p., mixed m.p., u.v. and i.r. spectra.

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[11] H. SCHMID and P. KARRER, *Helv. chim. Acta* **28**, 722 (1945).
[12] K. C. PANDYA and T. A. VAHIDY, *Proc. Indian Acad. Sci.* **4A**, 134 (1936); from *Chem. Abstr.* **320**, 8149 (1936).