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N-(*ρ*-COUMARYL)-TRYPTAMINE AND *N*-FERULYL-TRYPTAMINE IN KERNELS OF *ZEA MAYS**

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Key Word Index—*Zea mays*; Gramineae; sweet corn; *N*-(*ρ*-coumaryl)tryptamine; *N*-ferulyltryptamine; mass spectra.

Abstract—*N*-(*ρ*-coumaryl)tryptamine and *N*-ferulyltryptamine were isolated from aqueous acetone extracts of ground kernels of *Zea mays* by successive column chromatography on partially sulfonated styrenedivinylbenzene copolymer resin, lipophilic Sephadex and preparative TLC. Identification of these compounds was made by GC–MS of their trimethylsilyl derivatives and the trimethylsilyl derivatives of their acid hydrolysis products.

INTRODUCTION

CINNAMIC acid[1] and its hydroxy-, and hydroxymethoxy-derivatives, *ρ*-coumaric acid[2] and ferulic acid,[3] have been isolated from pineapple,[4] barley seeds and barley embryos,[5] members of the Cycadaceae,[6] and in trace amounts from etiolated oat coleoptiles, pea and sunflower seedlings.[7] Most of these phenolic acids exist in plants as esters of glucose,[7–13] as *β*-glucosides,[5,8] or as esters of quinic acid.[4,5,14–17] Tadera *et al.*,[18] and Oettmeier and Heupel,[19] have isolated a *ρ*-coumaryl derivative from spinach leaves and spinach leaf chloroplasts and shown it to be *ρ*-coumaryl-*meso*-tartaric acid.[20,21] Recently, Tanguy and

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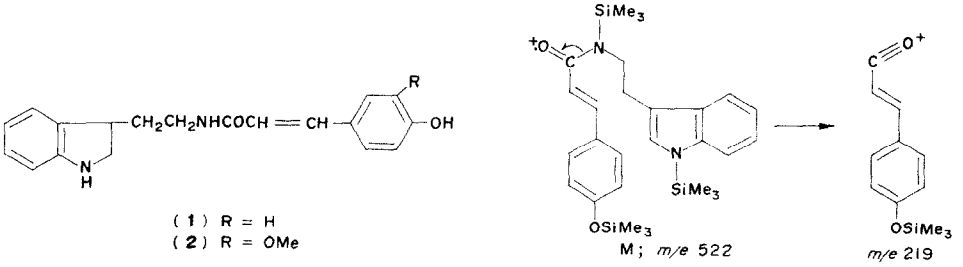
[1] DUMAS, J. and PELIGOT, E. (1834) *Justus Liebigs Ann. Chem.* **12,** 24.
[2] HLASIWETZ, H. (1865) *Justus Liebigs Ann. Chem.* **136,** 31.
[3] HLASIWETZ, H. and BARTH, L. (1866) *Justus Liebigs Ann. Chem.* **138,** 61.
[4] GORTNER, W. A., KENT, G. and SUTHERLAND, G. K. (1958) *Nature* **181,** 630.
[5] SUMERE VAN, C. F., COTTENIE, J., DEGREEF, J. and KINT, J. (1972) *Recent Advances in Phytochemistry*, Vol. 4, p. 165.
[6] WALLACE, J. W. (1972) *Am. J. Botany* **59,** 1.
[7] TOMASZEWSKI, M. (1964) *Colloq. Intern. Centre Natl. Rech. Sci. Paris* **123,** 335.
[8] HARBORNE, J. B. and CORNER, J. J. (1961) *Biochem. J.* **81,** 242.
[9] KOSUGE, T. and CONN, E. E. (1961) *J. Biol. Chem.* **236,** 1617.
[10] EL-BASYOUNI, S. Z., NEISH, A. C. and TOWERS, G. H. N. (1964) *Phytochemistry* **3,** 627.
[11] EL-BASYOUNI, S. Z. and NEISH, A. C. (1965) *Phytochemistry* **5,** 683.
[12] KOJIMA, M. and URITANI, I. (1972) *Plant Cell Physiol.* **13,** 1075.
[13] HARTLEY, R. D. (1973) *Phytochemistry* **12,** 661.
[14] BRADFIELD, A. F., FLOOD, A. E., HULME, A. C. and WILKINS, A. H. (1952) *Nature* **170,** 168.
[15] LEVY, C. C. and ZUCKER, M. (1960) *J. Biol. Chem.* **235,** 2418.
[16] BRAGT VAN, J., ROHRBAUGH, L. M. and WENDER, S. H. (1965) *Phytochemistry* **4,** 977.
[17] HANSON, K. R. (1966) *Phytochemistry* **5,** 491.
[18] TADERA, K., SUZUKI, Y., KAWAI, F. and MITSUDA, H. (1970) *Agr. Biol. Chem.* **34,** 517.
[19] OETTMEIER, W. and HUEPEL, A. (1972) *Z. Naturforsch.* **27b,** 177.
[20] TADERA, K. and MITSUDA, H. (1971) *Agr. Biol. Chem.* **35,** 1431.
[21] OETTMEIER, W. and HUEPEL, A. (1972) *Z. Naturforsch.* **27b,** 586.

Martin[22] have identified ferulyl- and caffeyl-malic acid in the cotyledons of bean. Amides of phenolic acids have also been found: *N*-caffeylputrescine,[23,24] *N*-(*p*-coumaryl)-2-hydroxyputrescine.[25] *N*-(feruloyl)-2-hydroxyputrescine,[25] *N,N,N'*-trimethyl-*N'*-(4-hydroxy-*cis*-cinnamyl)putrescine,[26] *N,N,N'*-trimethyl-*N'*-(4-methoxy-*cis*-cinnamyl)putrescine,[26] casimirvedine (*N*-cinnamyl-*N*-(glucosyl)histamine,[27,28] adenocarpin (*N*-cinnamyl)tetrahydroanabasin),[29] cinnamylhistamine,[30] *p*-coumarylagmatine (1-(*trans*-*p*-hydroxycinnamoylamino)-4-guanidinobutane,[31] and *N*-feruloylglycyl-L-phenylalanine.[32] The latter occurs as a peptide in barley globulins.

This report describes the isolation and identification from mature sweet corn kernels of two new phenolic acid derivatives in amide linkage with tryptamine, (**1**) and (**2**).



RESULTS AND DISCUSSION

Compounds (**1**) and (**2**) were isolated during the course of a study of esters of indole-3-acetic acid (IAA) and *myo*-inositol and *myo*-inositol glycosides[33] from kernels of *Z. mays* and purified by column chromatography on partially sulfonated styrene-divinylbenzene copolymer resin,[34] Sephadex LH20 and preparative TLC. Both compounds yielded a single spot on TLC and gave bluish-green colors with Ehrlich reagent. The R_f values were greater than the R_f value of IAA in two solvents (see Experimental), and the compounds did not yield IAA following mild alkaline hydrolysis. This indicated that (**1**) and (**2**) were not esters of IAA or related compounds. The trimethylsilyl (TMS) ethers of (**1**) and (**2**) were prepared as described before;[34] both compounds showed two peaks on GLC, a major first peak and a minor second peak, which permitted the direct analysis of (**1**) and (**2**) as their ethers by GC–MS. The MS of the first peak of the TMS-derivative of (**1**) showed a molecular ion (M^+) at *m/e* 522. The corresponding molecular ion of the direct probe analysis of the underivatized compound at *m/e* 306 (Table 1) shows that (**1**) has three TMS-groups. M^+ fragments to *m/e* 507 by the elimination of a methyl radical followed by the loss of a neutral $\cdot CH_2(CH_3)_2Si$ radical to give rise to *m/e* 435. The direct elimination of 72 a.m.u.

[22] TANGUY, J. and MARTIN, C. (1972) *C.r. Acad. Sci. Ser. D* **274**, 3402.
[23] HOLLERBACH, A. and SPITELLER, G. (1970) *Mh. Chem.* **101**, 141.
[24] MIZUSAKI, S., TANABE, Y. and NOGUCHI, M. (1970) *Agr. Biol. Chem.* **34**, 972.
[25] STOESSL, A., ROHRINGER, R. and SAMBORSKI, D. F. (1969) *Tetrahedron Letters* 2807.
[26] RIPPERGER, H., SCHREIBER, K. and BUDZIKIEWICZ, H. (1970) *F. Prakt. Chem.* **312**, 449.
[27] DJERASSI, C., BANKIEWICZ, C., KAPOOR, A. L. and RINIKER, B. (1958) *Tetrahedron* **2**, 168a.
[28] RAMAN, S., REDDY, J., LIPSCOMB, W. N., KAPOOR, A. L. and DJERASSI, C. (1962) *Tetrahedron Letters* 357.
[29] SCHUTTE, H. R., KELLING, K. L., KNOFEL, D. and MOTHES, K. (1964) *Phytochemistry* **3**, 249.
[30] FITZGERALD, J. S. (1964) *Australian J. Chem.* **17**, 375.
[31] STOESSL, A. (1965) *Phytochemistry* **4**, 973.
[32] SUMERE VAN, C. F., DEPOOTER, H., ALI, H. and VAN BUSSEL, D. (1973) *Phytochemistry* **12**, 407.
[33] UEDA, M., EHMANN, A. and BANDURSKI, R. S. (1970) *Plant Physiol.* **46**, 715.
[34] EHMANN, A. and BANDURSKI, R. S. (1972) *J. Chromatogr.* **72**, 61.

from the molecular ion predominates yielding the abundant ion at *m/e* 450. This elimination of 72 a.m.u. from the molecular ion has also been observed in the MS of (*N*-TMS-indole-3-acetyl)-*O*-penta-*O*-TMS-*myo*-inositol derivatives (unpublished results) and (*N*-TMS-indole-3-acetyl)-*O*-tetra-*O*-TMS-D-glucopyranose derivatives.[35] It involves the transfer of a hydrogen atom from one of the methyl groups of the *N*-TMS group to the indole ring with subsequent loss of the neutral $\cdot CH_2(Me)_2Si$ radical. This means that compound (**1**) must have two nitrogens (nitrogen rule) each of which is substituted with one TMS-group. The third TMS-group is on a hydroxyl oxygen. The loss of 202 a.m.u. from M^+ which gives rise to *m/e* 320, and the intense ions at *m/e* 202 and *m/e* 215 confirm the presence of *N*-substituted tryptamine.[36] The low intensity ions at *m/e* 130 and *m/e* 143 are the corresponding tryptamine fragments without the TMS-group on the imino-group. The presence of the high intensity ions *m/e* 130 and *m/e* 143 in the spectrum of the underivatized compound (**1**) (Table 1) support the structure proposed. A model compound 5-methoxy-*N*-acetyltryptamine (melatonin) was examined and found to yield analogous ion fragments at *m/e* 160, 232 and 245, thus confirming (**1**) as an *N*-acyl derivative of tryptamine.

TABLE 1. RELATIVE ION INTENSITIES (> 1·0%) IN THE MS OF THE UNDERIVATIZED COMPOUNDS (**1**) AND (**2**)*

	Compounds			Compounds	
m/e	(**1**)	(**2**)	*m/e*	(**1**)	(**2**)
336		5·0(M^+)	143	100·0	100·0
306	7·7(M^+)		130	51·7	68·5
194		3·8	119	16·2	1·7
177		24·1	117	1·9	14·1
164	7·7		103	3·8	14·4
159	27·4	1·9	91	12·7	6·1
149		8·4	89	10·8	17·7
147	26·6	2·4	77	54·8	26·8

* The 70 eV MS were obtained by introducing 500 ng of (**1**) and (**2**) directly into the ion source via a direct probe inlet at a probe temp. of 125°.

The ion at *m/e* 219 (or its analogue *m/e* 147, Table 1) arises from M^+ by loss of the neutral tryptamine fragments with the structure with charge retention on the acyl group. This ion further fragments to give *m/e* 203, a part of which is the isotope of *m/e* 202. This transition is supported by the presence of a small metastable peak at *m/e* 188·1 (calculated: 188·1). The fragment *m/e* 219 also gives rise to the ion *m/e* 191 by the loss of CO, a transition supported by the small metastable peak found at *m/e* 166·5 (calculated: 166·6). Horman and Viani[37] have described the same series of eliminations from *m/e* 219 for the fragmentation of the TMS-derivatives of *m*-coumaric and *p*-coumaric acid, suggesting that the acyl group of compound (**1**) could be a hydroxycinnamic acid. The presence of the ion *m/e* 249, a characteristic rearrangement ion in the mass spectra of the TMS-ethers of hydroxy-, and hydroxy-methoxycinnamic acids,[37] supports this conclusion. The ion *m/e* 249 is not present in the mass spectrum of the TMS-derivative of *o*-coumaric acid[37] which suggests that the acyl group in compound (**1**) is either a *m*-coumaryl or *p*-coumaryl group.

The mass spectrum of the second peak of the TMS-derivative of compound (**1**) with a free amide nitrogen supports the structure proposed for (**1**).

[35] EHMANN, A. (1974) *Carbohyd. Res.* **33**, In press.
[36] NARASHIMHACHARI, N. SPAIDE, J. and HELLER, B. (1971) *J. Chromatogr. Sci.* **9**, 502.
[37] HORMAN, I. and VIANI, R. (1971) *Org. Mass Spectrom.* **5**, 203.

To obtain additional evidence concerning the identity of the acyl group, compound (**1**) was hydrolyzed with 2·0 M trifluoroacetic acid (TFA) and the hydrolysis products analyzed by GLC and combined GLC–MS as the TMS-ethers. Two compounds were found which on GLC cochromatographed with TMS-tryptamine and TMS-*p*-coumaric acid. Their MS were identical with the MS of tryptamine and *p*-coumaric acid. Compound (**1**) is thus identified as *N*-(*p*-coumaryl)tryptamine.

The MS of the first peak of the TMS-derivative of (**2**) showed a molecular ion at *m/e* 552, and the corresponding molecular ion of direct probe analysis of the underivatized compound is at *m/e* 336 (Table 1). The presence of the ions *m/e* 215, 202, 130 and 143 identify the indole as an *N*-substituted tryptamine. The difference of 30 a.m.u. between compounds (**1**) and (**2**) indicates the presence of a methoxy group on the acyl moiety. This is confirmed by the shift of 30 a.m.u. of *m/e* 320 and *m/e* 450 of compound (**1**) to *m/e* 350 and *m/e* 480. The presence of a strong intensity ion at 249 (or its analogue *m/e* 177, Table 1) with a concomitant low intensity ion at *m/e* 219, is diagnostic of TMS-derivatives of ferulic and isoferulic acid.[37] The acyl group of compound (**2**) is therefore either a feruloyl- or *iso*-feruloyl group. The MS of the second peak of the TMS-derivative of compound (**2**) with its free amide nitrogen supports this conclusion.

GLC analysis of the acid hydrolysis products of compound (**2**) as their TMS-ethers disclosed two compounds which co-chromatographed with authentic TMS-ferulic acid and tryptamine. Combined GLC–MS yielded mass spectra identical with those obtained from the authentic TMS-acid and tryptamine. Compound (**2**) is therefore identified as *N*-feruloyltryptamine. The only other known acyltryptamine, behenoyltryptamine has been isolated from cocoa shells.[38]

EXPERIMENTAL

Isolation of compounds (**1**) *and* (**2**). Extraction of 10 kg of ground kernels of *Zea mays* L. (cultivar, Stowell's Evergreen hybrid) was carried out as previously described.[33,34] The resulting *n*-BuOH phase was dried at reduced pressure at 45°, the residue taken up in 40 ml of H_2O, and the H_2O-insoluble fraction removed by filtration. The H_2O-insoluble fraction was dissolved in 10 ml of 50% EtOH and chromatographed on a 20% sulfonated[34] styrene divinylbenzene co-polymer resin (column i.d. 9·0 mm, bed vol. 38·2 ml, void vol. 13·5 ml) with 50% EtOH as eluent, and collecting 2·0 ml fractions. Compounds (**1**) and (**2**) emerged between 0·6 and 2·9 bed vol. Small aliquots of each fraction (10–50 μl) were subjected to TLC[34] and the fractions containing the Ehrlich-positive compounds (**1**) and (**2**) were pooled. The pooled fractions were dried, redissolved in 4·0 ml of 50% EtOH and chromatographed on Sephadex LH-20 (column i.d. 9·0 mm, bed vol. 38·5 ml, void vol. 8·5 ml) using 50% EtOH as eluent. Fractions of 1·5 ml were collected and monitored on TLC. Compound (**1**) eluted between 2·7–3·5 bed vol., and compound (**2**) between 3·5–4·3 bed vol. Both compounds were further purified by preparative TLC using: MeCOEt–EtOAc–EtOH–H_2O (3:5:1:1) and $CHCl_3$–MeOH–H_2O (85:14:1). Relative R_f values (R_f of IAA = 1) of (**1**) and (**2**) were 1·02 and 1·02, and 1·95 and 1·80 respectively. The total amount of (**1**) obtained was 1400 μg/10 kg (140 μg/kg) and 400 μg/10 kg (40 μg/kg) of (**2**) as determined colorimetrically with Ehrlich reagent and quantitative GLC of the TMS-derivatives.

Acid hydrolysis of compounds (**1**) *and* (**2**). Small samples (20–50 μg) of both compounds were hydrolyzed in 2·0 M TFA in sealed ampules at 50° for 2 hr. This was found to give about 50% hydrolysis with a minimum loss of the phenolic acid fractions.[5] TFA was used since it is volatile and could be removed to permit derivatization of the hydrolysis products for GLC and GLC–MS.

GC–MS. The TMS-derivatives of compounds (**1**) and (**2**), their hydrolysis products and the standards melatonin, tryptamine, *p*-coumaric acid and ferulic acid[39,40] were prepared as described before.[34] The TMS-ethers were chromatographed on a F and M model 402 gas chromatograph equipped with flame ionization detectors, and using nitrogen as carrier gas at a flow rate of 60 ml/min on a 1·2 m × 3·0 mm i.d. glass column packed with OV-1, 2% on gas chrom Z (100/120 mesh). GC–MS was performed on a LKB 9000 mass spectrometer with a 1·2 m × 3·0 mm i.d. glass column packed with OV-1, 2% on gas chrom Z (100/120 mesh) and helium as a carrier

[38] SACHER, H. (1965) *Z. Lebensur. Untersuch. Forsch.* **128**, 264.
[39] DALLAS, F. C. and KOEPPL, K. G. (1969) *J. Chromatogr. Sci.* **7**, 565.
[40] STEELE, J. W. and BOLAN, M. (1972) *J. Chromatogr.* **71**, 427.

gas at a flow rate of 25 ml/min. The ionizing energy was 70 eV, the flash heater 250°, the molecular separator 250° and the ion source temperature 290°. The mass sectra were recorded using an on-line data acquisition and processing program.[41] MS of the underivatized compounds (**1**) and (**2**) were also recorded using a solid probe in-let system.

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[41] SWEELEY, C. C. (1973) *Introduction to Lipid Chemistry* (BURTON, R. M., ed.), Webster Groves, Mo., in press.