Synthesis of Fagaronine. An Anticancer Benzophenanthridine Alkaloid

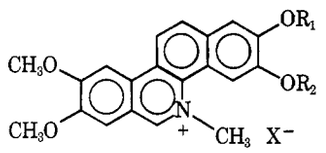
John P. Gillespie, Luis G. Amoros, and Frank R. Stermitz*

Department of Chemistry, Colorado State University, Fort Collins, Colorado 80521

Received May 29, 1974

A structural proof for fagaronine has been attained by synthesis. The sequence employed represents a new approach to the synthesis of such alkaloids by use of the Kessar benzyne cyclization. Various phenolic protecting groups were explored, of which the isopropoxy group was found to be most compatible with the overall synthetic scheme.

Recently, an extremely active antileukemic alkaloid, fagaronine, was isolated[1] from *Fagara zanthoxyloides* (Rutaceae). The structure of fagaronine was left somewhat in doubt, however, as spectral methods were insufficient to absolutely distinguish between **1a** and **1b,** although **1a** was suggested[1] as the more likely structure.



1a, $R_1 = H$; $R_2 = CH_3$; $X = Cl^-$
b, $R_1 = CH_3$; $R_2 = H$; $X = Cl^-$
c, $R_1 = H$; $R_2 = CH_3$; $X = CH_3OSO_3^-$

We wish to report a short synthesis of fagaronine which chemically establishes its structure unambiguously, supporting Farnsworth's recent[2] spectroscopic structural proof. The basis of the method is the Kessar phenanthridine synthesis,[3] which has been applied for the first time for the preparation of benzophenanthridine alkaloids.

Results and Discussion

Our synthesis is outlined in Scheme I. The key transformation in this synthetic approach is the cyclization of the anil **6.** Attempts to cyclize **6a** failed completely, presumably because of the presence of a free phenolic group. Possible reasons for such failures have been discussed by Kessar.[3]

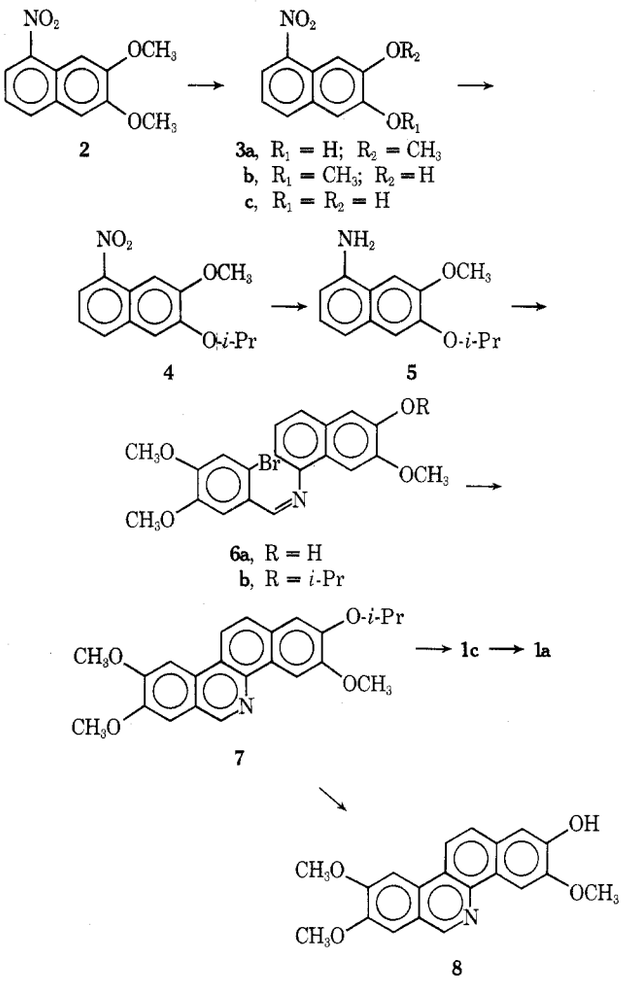
Therefore, a series of protecting groups was examined. The choice of a suitable group unexpectedly proved to be the most imposing problem of the synthesis. With model compounds seven different phenolic protecting groups were investigated. Six were discarded for the reasons shown in Table I. Although some limited success was achieved with the benzyl group in model compounds, only the isopropoxy group[4] possessed none of the undesirable properties listed in Table I.

Nitration of 2,3-dimethoxynaphthalene with fuming nitric acid in acetic acid gave all three possible nitro isomers as reported[5] by Chang, Moore, and Scheuer. However, we have circumvented the laborious chromatographic separation procedure[5] by the use of suitable crystallization solvents, from which all three isomers can be obtained in pure form. The progress of the successive fractional crystallizations was monitored by liquid chromatography.[6a]

Cleavage of **2** carried to 50% completion gave a 1:1 mixture[6b] of **3a** and **3b,** which were readily separated by crystallization. The compound **3a** is known,[7] being the exclusive product of the photolysis of **2** in CH_3CN–0.8 *N* NaOH in H_2O. The photolysis procedure[7] was found to be inconvenient for larger scale preparative work.

The nitrophenol **3a** was smoothly isopropylated in DMF and isopropyl bromide in the presence of K_2CO_3.[8] Reduction of the nitro groups in 4 was achieved by Pd/C and hydrazine in ethanol,[9] giving the naphthylamine **5** in high yield. Condensation of **5** with *o*-bromoveratraldehyde proceeded rapidly to give the anil **6b.** Cyclization of the anil under the Kessar conditions[3] ($NaNH_2$ in liquid NH_3) gave the extremely insoluble benzophenanthridine **7.**

Scheme I



Originally, we had planned to cleave the isopropoxy group to give **8** and then N-alkylate the phenolic free base. Indeed, the isopropoxy group was easily and selectively removed in the presence of the methoxy functionalities[4] in HBr–HOAc at 100°. Unfortunately, 8 alkylated poorly and inconsistently under the reaction conditions (40–65% conversion was typical). Further, the free base 8 is difficult to separate from **1c.**

However, N-alkylation of **7** directly gave reasonable conversion. Isolation of the mixture and reexposure to the reaction conditions (dimethyl sulfate, xylene, nitrobenzene, 180°) cleaved the isopropoxy group and a careful work-up

Table I
Protecting Groups ArOR

R	Reason for rejection
H	*b, d, f*
CH_2OCH_3	*b, e, f*
$SO_2C_6H_5$	*g*
$SO_2C_6H_4NO_2$-*p*	*c*
$CH_2C_6H_5$	*a, e, f*
CO_2Me	*c*
$CH_2CH_2C_6H_5$	*b, c*

[a] Renders extreme lability to the intermediate naphthylamine. [b] Interferes with the anil formation reaction. [c] Unstable to liquid NH_3–$NaNH_2$. [d] Prevents cyclization (see text). [e] Renders the product free base soluble (insolubility is important for the isolation step). [f] Interferes with N-alkylation. [g] Could not be removed after completion of the N-alkylation step.

(see Experimental Section) gave a fair conversion to **1c**. Exchange of the methosulfate conterion for chloride in aqueous NaCl[10] yielded a sample of **1a** identical in all respects with authentic fagaronine. Work in progress indicates that this synthetic method is adaptable to the synthesis of many benzophenanthridine alkaloids in sufficient amounts for possible preclinical or clinical testing.

Experimental Section

5-Nitro-2,3-dimethoxynaphthalene (2). The procedure of Bell and Buck[11] was followed for the nitration. A solution of 150 g of 2,3-dimethoxynaphthalene in 1800 ml of acetic acid was treated with a mixture of 150 ml of fuming nitric acid in 150 ml of acetic acid. After stirring for 50 min, the mixture was poured into 3 l. of cold water and extracted twice with a total of 1100 ml of $CHCl_3$. The organic layer was extracted with 2 l. of 12.5% KOH. A green solid formed and the emulsion was filtered. The organic extract was combined with an additional washing of 100 ml of $CHCl_3$ and dried. Removal of the solvent gave 157.5 g of a mixture of the 1-, 5-, and 6-nitro isomers. The crude residue was dissolved in 2400 ml of 95% ethanol and allowed to stand overnight. Filtration gave 65 g of the 5 and 6 isomers uncontaminated by the 1 isomer. Further crystallization of this mixture from ethyl acetate or acetic acid gave pure **2**, mp 158–159° (lit.[5] mp 157–158°).

2-Hydroxy-3-methoxy-5-nitronaphthalene (3a). Method A. Photolysis according to Havinga[7] of a solution of 700 mg of **2** in 5 l. of a mixture of 65% CH_3CN and 35% 0.8 *N* NaOH through a Pyrex filter with a 450-W Hanovia lamp gave, after 1 hr, a deep red solution. The CH_3CN was removed by rotary evaporation and the basic solution was extracted several times with $CHCl_3$. The aqueous layer was acidified and again extracted with $CHCl_3$. The latter extract was dried, the solvent was removed, and the residue was crystallized from ethanol–water to yield 550 mg (90%) of **3a**: mp 126–127° (no literature melting point given); nmr ($CDCl_3$) δ 4.12 (s, OCH_3), 6.15 (s, OH), 7.33 (s, H_4), 7.35 (t, J = 8 Hz, H_6), 7.92 (d of d, J = 1.5, 8 Hz, H_5), 8.05 (s, H_1), 8.17 (d of d, J = 1.5, 8 Hz, H_7); uv (EtOH) λ_{max} 357 nm (log ϵ 3.65), 222 (4.85); ir ($CHCl_3$) 3542 (OH), 1515 and 1345 cm^{-1} (NO_2).

Anal. Calcd for $C_{11}H_9NO_4$: C, 60.27; H, 4.13; N, 6.39. Found: C, 60.43; H, 4.07; N, 6.33.

Method B. A solution of 20 g of **2** in 500 ml of HOAc and 100 ml of 48% HBr was heated at 100° for 3 hr, which brought the reaction to approximately 50% completion. The solution was poured into 2.0 l. of H_2O and extracted several times with $CHCl_3$. The organic extract was washed several times with water to remove small amounts of **3c** formed as a by-product of the reaction. Shaking with 10% NaOH formed a deep red aqueous layer which was washed several times with $CHCl_3$. The combined organic layers yielded the neutral fraction of **2**, mp 155–157°. Acidification of the aqueous layer and extraction with $CHCl_3$, drying, and solvent removal gave the phenolic products as an approximate 1:1 mixture of **3a** and **3b**. Fractional crystallization alternately from benzene and CH_3CN gave pure samples of the two isomers: **3a**, 1.5 g, mp 124.5–126° (CH_3CN); **3b**, 3.7 g, mp 159–160° (benzene), nmr ($CDCl_3$) δ 4.10 (s, $-OCH_3$), 6.22 (s, OH), 7.25 (s, H_4), 7.38 (t, J = 8 Hz, H_6), 8.0 (d of d, J = 2, 8 Hz), H_5), 8.18 (s, H_1), 8.20 (d of d, J = 2, 8 Hz, H_7).

Anal. Calcd for $C_{11}H_9NO_4$: C, 60.27; H, 4.13; N, 6.39. Found: C, 60.32; H, 4.29; N, 6.40.

2-Isopropoxy-3-methoxy-5-nitronaphthalene (4). To 5 g of **3a** in 10 ml of DMF was added 10 g of anhydrous K_2CO_3.[8] Isopropyl bromide (10 ml) was added and the mixture was heated at 100°. After 2.5 hr the red color faded and was replaced by a yellow precipitate. The mass was poured into water and extracted with $CHCl_3$. The organic layer was extracted several times with water to remove the DMF, then dried and evaporated. Recrystallization of the residue from ethanol yielded 5.5 g (92%) of **4**: mp 109–110°; nmr ($CDCl_3$) δ 1.48 [d, J = 6 Hz, $-CH(CH_3)_2$], 4.05 (s, $-OCH_3$), 4.78 [septet, J = 6 Hz, $-CH(CH_3)_2$], 7.23 (s, H_1), 7.43 (t, J = 8 Hz, H_6), 7.95 (d of d, J = 2, 8 Hz, H_5), 8.08 (s, H_4), 8.18 (d of d, J = 2, Hz, H_7); uv (EtOH) λ_{max} 357 nm(log ϵ 3.75), 260 (sh) (4.04), 223 (4.79); ir ($CHCl_3$) 1510 and 1339 cm^{-1} (NO_2).

Anal. Calcd for $C_{14}H_{15}NO_4$: C, 64.36; H, 5.78; N, 5.36. Found: C, 64.68; H, 5.58; N, 5.31.

2-Isopropoxy-3-methoxy-5-aminonaphthalene (5). The general procedure[9] of Dewar and Mole was used. Ethanol (100 ml), hydrazine (5 ml), **4** (5.0 g), and 10% Pd/C (0.5 g) were heated on a steam bath for 1 hr. Filtration through a filter cell removed the Pd/C and evaporation of the ethanol and hydrazine with the aid of benzene gave 4.4 g (99%) of a viscous oil which, after standing overnight, contained a few tiny crystals. Addition of ether induced complete crystallization. Sublimination gave an analytical sample: mp 88–89°; nmr ($CDCl_3$) δ 1.37 [d, J = 6 Hz, $-CH(CH_3)_2$], 3.81 (s, $-OCH_3$), 3.91 (broad s, $-NH_2$), 4.63 [septet, J = 6 Hz, $-CH(CH_3)_2$], 6.55 (d of d, J = 4, 5 Hz, 1 H), 6.95–7.18 (m, 4 H); uv (EtOH) λ_{max} 308 nm (log ϵ 3.48), 254 (4.57), 241 (4.36), 222 (4.52); ir ($CHCl_3$) 3470 and 3395 cm^{-1} (NH_2).

Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.72; H, 7.36; N, 6.06. Found: C, 72.45; H, 7.51; N, 5.89.

2-Bromo-4,5-dimethoxybenzal-2′-isopropoxy-3′-methoxy-5-naphthylamine (6b). A 100-ml benzene solution of **5** (4.0 g) and *o*-bromoveratraldehyde (4.24 g) was refluxed with a Dean-Stark trap until water evolution ceased. Removal of the benzene gave a yellow solid. Trituration with ether and filtration yielded 7.45 g (91%) of **5**, mp 150–152°. An analytical sample crystallized from ethyl acetate had mp 153–154°; nmr ($CDCl_3$) δ 1.46 [d, J = 6 Hz, $-CH(CH_3)_2$], 3.96 (s, OCH_3), 4.06 (s, 2-OCH_3), 4.76 [septet, J = 6 Hz, $-CH(CH_3)_2$], 6.90–7.60 (m, 5 H), 7.70 (s, 1 H), 8.0 (s, 1 H), 9.25 (s, –CH=N–); uv (EtOH) λ_{max} 358 nm (log ϵ 3.86), 332 (3.91), 280 (4.20), 246 (sh) (4.73), 217 (4.79); ir ($CHCl_3$) 1621 cm^{-1} (C=N).

Anal. Calcd for $C_{23}H_{24}BrNO_4$: C, 60.26; H, 5.27; N, 3.05. Found: C, 60.09; H, 5.17; N, 2.75.

2-Isopropoxy-3,8,9-trimethoxybenzo[*c*]phenanthridine (7). Cyclization of **6** was by the method of Kessar.[3] To a suspension of sodium amide (from 2.10 g of Na) in liquid ammonia was added 7.0 g of **6b** as a finely pulverized powder. After 0.5 hr, ammonium chloride was added until the red-brown color of the ammonia changed to yellow. Work-up of the residue after evaporation of the NH_3 with $CHCl_3$ and water gave, after drying and evaporation of the organic layer, a dark yellowish-brown oil. Addition of 200 ml of ethanol and boiling on a steam bath caused precipitation of a yellow solid. Filtration yielded 1.38 g (24%) of **7**, mp 264–266°. An analytical sample crystallized from CH_3NO_2 had mp 270–272°; nmr (CD_3CO_2D) δ 1.49 [d, J = 6 Hz, $CH(CH_3)_2$], 4.09 (s, OCH_3), 4.13 (s, OCH_3), 4.24 (s, OCH_3), 4.90 [septet, J = 6 Hz, $-CH(CH_3)_2$], 7.41 (s, 1 H), 7.67 (s, 1 H), 7.87 (s, 1 H), 7.98 (d, J = 9 Hz, 1 H), 8.22 (s, 1 H), 8.29 (d, J = 9 Hz, 1 H), 9.47 (s, 1 H); uv (EtOH) λ_{max} 328 nm (log ϵ 4.04), 310 (sh) (4.36), 285 (4.80), 275 (4.80); ir ($CHCl_3$) 1623 cm^{-1} (–C=N–).

Anal. Calcd for $C_{23}H_{23}NO_4$: C, 73.19; H, 6.14; N, 3.71. Found: C, 73.05; H, 6.24; N, 3.74.

2-Hydroxy-3,8,9-trimethoxybenzo[*c*]phenanthridine (8). To a solution of **7** (200 mg) in 15 ml of HOAc was added 1 ml of 48% HBr. After heating for 3 hr, tlc indicated the absence of **7**. The mixture was poured into water, neutralized to pH 8, and extracted with $CHCl_3$. Evaporation of the dried solvent gave 160 mg (90%) of a tan solid. Crystallization from EtOAc gave a sample: mp 274–276°; nmr[2] (DMSO-d_6) δ 4.05 (s, OCH_3), 4.12 (s, OCH_3), 4.17 (s, OCH_3), 7.38 (s, H_1), 7.70 (s, H_7 or H_{10}), 7.86 (d, J = 9 Hz, H_{12}), 8.15 (s, H_7 or H_{10}), 8.55 (d, J = 9 Hz, H_{11}), 8.68 (s, H_4), 9.35 (s, H_6); uv (EtOH) λ_{max} 228 nm (log ϵ 4.21), 27 (5.02), 283 (5.03), 315 (4.45); ir ($CHCl_3$) 3510 (OH), 1624 cm^{-1} (C=N).

Fagaronine Methosulfate (1c). A 400-mg sample of **7** was heated at 180° in 8 ml of nitrobenzene and 4 ml of xylene. Dimethyl sulfate (1 ml) was added down the condenser. The mixture darkened immediately and heating was continued for 7 min. After cooling, ether was added and the precipitate (380 mg) was collected by

filtration. An nmr spectrum in DMSO-d_6 indicated approximately 70% alkylation and that the isopropoxy group was still present. The entire sample was reexposed to the above reaction conditions. After heating for 10 min, the mixture was allowed to cool. The precipitate of about 200 mg was collected by filtration and shown by nmr to be a 1:1 mixture of **8** and **1c**. The filtrate was added to 25 ml of ether and the brownish precipitate was collected (150 mg). Tlc analysis indicated the absence of any of the free base **8**. Recrystallization of this material from methanol gave 95 mg of pure **1c**: mp >350° dec, softens at 220°; nmr[12] (DMSO-d_6) δ 3.77 (s, $-OSO_3CH_3$), 4.08 (s, OCH_3), 4.15 (s, OCH_3), 4.26 (s, OCH_3), 5.03 (s, NCH_3), 7.63 (s, H_1), 7.97 (s, H_7), 8.23 (s, H_4), 8.25 (d, J = 9 Hz, H_{12}), 8.35 (s, H_{10}), 8.85 (d, J = 9 Hz, H_{11}), 9.99 (s, H_6).

Anal. Calcd for $C_{22}H_{23}NO_8S \cdot H_2O$: C, 55.11; H, 5.22; N, 2.92. Found: C, 54.97; H, 5.04; N, 2.97.

Fagaronine Chloride (1a). The exchange of counterions was by the method of Zee Cheng and Cheng.[10] In an 8% aqueous NaCl solution (12 ml) was suspended 50 mg of **1c**. After stirring for 0.5 hr at room temperature, filtration gave 36 mg (88%) of **1a**, mp 198–200°, with resolidification and remelting at 260–261° (lit.[1] 202°, 255°). The nmr, ir, uv, and base-shifted uv were identical with those of an authentic sample.[2]

Acknowledgment. This work was supported in part by Grants CA 13648 from the National Cancer Institute and GM 15425 from the National Institute of General Medical Sciences, National Institutes of Health.

Registry No.—**1a**, 52259-64-0; **1c**, 52259-66-2; **2**, 7311-22-0; **3a**, 24309-45-3; **3b**, 52259-67-3; **4**, 52259-68-4; **5**, 52259-69-5; **6b**, 52259-70-8; **7**, 52259-71-9; **8**, 52259-72-0; *o*-bromoveratraldehyde, 5392-10-9.

References and Notes

(1) W.M. Messmer, M. Tin-Wa, H.H.S. Fong, C. Bevelle, N.R. Farnsworth, D.J. Abraham, and J. Trojanek, *J. Pharm. Sci.*, **61**, 1858 (1972).
(2) During the course of this work, we were informed (M. Tin-Wa, C.L. Bell, C. Bevelle, H.H.S. Fong, and N.R. Farnsworth, *J. Pharm. Sci.*, in press) that fagaronine had been converted to the *N*-demethyl base (identical with **8**) whose spectral properties clearly established the fagaronine as **1a**. We thank Dr. Farnsworth for a preprint of his work and for a standard sample of fagaronine.
(3) S.V. Kessar, D. Pal, and M. Singh, *Tetrahedron*, **29**, 167 (1974).
(4) T.H. Simpson, *J. Org. Chem.*, **28**, 2107 (1963).
(5) C.W. Chang, R.E. Moore, and P.J. Scherer, *J. Chem. Soc. C*, 840 (1967).
(6) (a) A Waters ALC 202 (uv detector) fitted with a 2 ft × 0.375 in. Porasil column gave clean separation with 80:20 cyclohexane–chloroform for analytical purposes. (b) A 2 ft × 0.375 in. Corasil column was used.
(7) G.M. Beijersbergen Van Henegowen and E. Havinga, *Recl. Trav. Chim. Pays-Bas*, **89**, 909 (1970).
(8) P. Hackney and T. Nestrick, *J. Chem. Eng. Data*, **13**, 881 (1968).
(9) M.J.S. Dewar and T. Mole, *J. Chem. Soc.*, 2556 (1956).
(10) K.Y. Zee Cheng and C.C. Cheng, *J. Heterocycl. Chem.*, **10**, 85 (1973).
(11) F. Bell and K.R. Buck, *J. Chem. Soc. C*, 904 (1966).
(12) Proton assignments by analogy to nitidine chloride: R.A. Labriola, A.M. Kuck, and J. Comin, *An. Asoc. Quim. Argent.*, **54**, 29 (1966).

7-Hydroxymyoporone, a New Toxic Furanosesquiterpene from Mold-Damaged Sweet Potatoes

Leo T. Burka,* Robert M. Bowen, and Benjamin J. Wilson

Center in Environmental Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

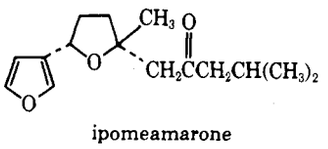
Thomas M. Harris

Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235

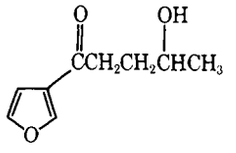
Received May 21, 1974

A new stress metabolite of the sweet potato has been isolated and identified as 1-(3-furoyl)-4,8-dimethyl-7-hydroxy-1,6-nonanedione (7-hydroxymyoporone). The structure assignment was based on spectral data and transformation to 3-methyl-5-(3-furoyl)pentanoic acid. The degradation product was synthesized by a reaction sequence involving the acylation of methyl 3-methyl-5-oxocyclopentanecarboxylate with 3-furoyl chloride and cleavage of the resulting diketo ester with aqueous base. The toxicity of 7-hydroxymyoporone is similar to that of the well-known sweet potato phytoalexin, ipomeamarone.

Sweet potatoes, when infected by fungus or when subjected to certain other stress conditions, elaborate numerous furanoid metabolites, all of apparent terpenoid origin.[1] Ipomeamarone was the first of these to be isolated



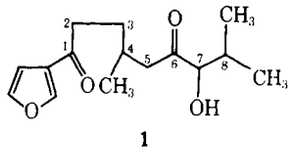
ipomeamarone



4-ipomeanol

and identified.[2] The compound is hepatotoxic and is usually the most abundant of the furan metabolites. We have been particularly interested in a group of 1,4-dioxygenated 1-(3-furyl)pentanes, also isolated from mold-damaged sweet potatoes.[3] These compounds, especially 1-(3-furyl)-4-hydroxy-1-pentanone (4-ipomeanol), show a marked specific pulmonary toxicity in laboratory animals.

As part of our continuing investigation of the phytoalexins of the sweet potato, we have isolated a new furanosesquiterpene which has been identified as 1-(3-furyl)-7-hydroxy-4,8-dimethyl-1,6-nonanedione (7-hydroxymyoporone, **1**).[4] The compound was obtained from sweet potato slices that had been incubated with cultures of *Ceratocystis fimbriata*.[5] The isolation involved extraction into ethyl acetate and chromatography on silica gel followed by preparative glpc of the partially purified material, after treatment with a trimethylsilylating reagent. The trimethylsilyl ether was cleaved with tetra-*n*-butylammonium fluoride[6] to give the pure metabolite in a yield of 20 mg/kg of sweet potatoes (wet weight).



1