after two recrystallizations from ethanol. The semicarbazone of PGA_2, which to our knowledge is the first reported crystalline derivative of this substance, could be prepared in 90% yield by reaction with semicarbazide at pH 3 in a 1:1 mixture of ethanol–water (mp 124.5–126° from ethanol).[8]

In contrast to prostaglandin synthetases isolated from mammalian sources,[9] no cofactors have as yet been found for this prostaglandin synthetase. Glutathione, cysteine, coenzyme A, thioglycolic acid, and mercaptoethanol, each at 10^{-3} *M* concentration, produced 20–80% inhibition. A concentration of 10^{-3} *M* EDTA or citric acid had no effect; the addition of 10^{-2} *M* Ca^{2+} or Mg^{2+}, 10^{-4} *M* Zn, V, Co, or Fe, or 10^{-5} *M* Cu was also without effect. Addition of ATP (10^{-3} *M*) or NADH or NADPH (5×10^{-4} *M*) also had no effect. It is interesting that strong inhibition of the mammalian synthetase by Cu^{2+} and Zn^{2+} at these concentrations has been noted.[9a]

The mammalian and gorgonian-derived prostaglandin synthetases show remarkably different behavior toward other inhibitors. Thus, indomethacin is a potent inhibitor of the mammalian synthetase[10] (complete inhibition at 2.5×10^{-5} *M*) but displays no such interaction toward the synthetase from *P. homomalla* (no observable inhibition at 2.5×10^{-5} *M*). Similarly, no inhibition was noted with 5,8,11,14-eicosatetraynoic acid (6×10^{-5} *M*), a powerful inhibitor of the mammalian prostaglandin synthetase.[11]

As expected, the gorgonian synthetase could also utilize 8,11,14-eicosatrienoic acid as substrate. Under the same conditions which allow the biosynthesis of PGA_2 from arachidonic acid, enzymic conversion of 2-tritio-8,11,14-eicosatrienoic acid to labeled PGA_1 was observed.

Since PGE_2 had been reported to be present in the *Plexaura homomalla*, we felt that the PGA_2 should be produced from the intermediary PGE_2.[2,3] Surprisingly, incubations with labeled PGE_1 did not result in labeled PGA_1. Likewise, incubations of 2-tritio-8,11,14-eicosatrienoic acid in the presence of 1 mg of cold PGE_1 resulted in only labeled PGA_1 formation. No labeled PGE_1 appeared. These results suggest that any intermediary PGE must be bound tightly to the enzyme complex. Furthermore, the enzyme responsible for converting PGE to PGA must not be accessible to exogenous PGE.

Our initial efforts to purify the PGA_2 synthetase of *P. homomalla* have thus far been foiled. Enzymic activity is lost upon storage at 0° for 24 hr, attempted fractionation by either ammonium sulfate or acetone (−20°) precipitation, or column chromatography.

Our investigations of prostaglandin biosynthesis are continuing.

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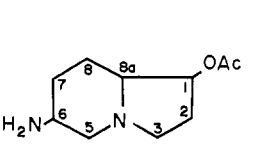
Isolation and Characterization of a 1-Pyrindine Fungal Alkaloid[1]

Sir:

The fungus *Rhizoctonia leguminicola* utilizes the entire carbon skeleton of pipecolic acid (derived from the metabolism of lysine)[2] in the biosynthesis of the parasympathomimetic alkaloid slaframine (**1**, (1*S*,6*S*,8a*S*)-1-acetoxy-6-aminooctahydroindolizine).[3–5] We now report the isolation and characterization of a related alkaloid produced by this fungus. We have assigned to it structure **2** (3,4,5-trihydroxyoctahydro-1-pyrindine). Apparently, this is the first example of a 1-pyrindine isolated from a biological source.



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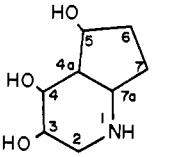

2

Tritiated **2** (biosynthesized from [^{3}H]pipecolic acid[2b]) was isolated from ethanolic mycelial extracts by ion-exchange[6] and preparative thin-layer chromatographies (silica gel G; $CHCl_3$–CH_3OH–4% NH_4OH, 40:40:20; acetone–$CHCl_3$–50% aqueous diethylamine, 60:20:40). The purified material appeared as a single spot in four different thin-layer systems (detection: iodine, uv, ninhydrin) and as a single peak on gas–liquid chromatography (3% OV-17, 185°). The yield of pure **2** was 50 mg/4 kg wet weight of mycelia. Milder procedures produced the same compound, showing that it is a true metabolite and not an artifact arising during isolation.

The molecular formula $C_8H_{15}NO_3$ (*m/e* 173.1060) was obtained by high-resolution mass spectrometry. Strong hydroxyl absorption (3350 cm^{-1}) was present in the infrared (KBr, $CHCl_3$). The compound was unaffected by treatment with $NaBH_4$ or by attempted acid or base hydrolysis; no unsaturation was indicated by either nmr or ir. Thus, **2** is bicyclic. The presence of a secondary amine was indicated by positive reaction

(1) Partial reports of the present work: (a) F. P. Guengerich, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **30**, 1067 (1971); (b) F. P. Guengerich, S. J. DiMari, and H. P. Broquist, 164th National Meeting of the American Chemical Society, New York, N. Y., Aug 27–Sept 1, 1972, Abstract No. BIOL 226.

(2) (a) H. P. Broquist and J. J. Snyder in "Microbial Toxins," S. Kadis, A. Ciegler, and S. J. Ajl, Ed., Vol. 7, Academic Press, New York, N. Y., 1971, p 319; (b) F. P. Guengerich, J. J. Snyder, and H. P. Broquist, manuscript in preparation.

(3) F. P. Guengerich and H. P. Broquist, manuscript in preparation.

(4) (a) D. Cartwright, R. A. Gardiner, and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.*, **92**, 7615 (1970); (b) R. A. Gardiner, K. L. Rinehart, Jr., J. J. Snyder, and H. P. Broquist, *ibid.*, **90**, 5639 (1968); (c) S. D. Aust, H. P. Broquist, and K. L. Rinehart, Jr., *ibid.*, **88**, 2879 (1966).

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(8) The pH of the reaction is extremely critical. Little or no crystalline semicarbazone of PGA_2 could be obtained at pH 4 or 5 or pH 2. The semicarbazone of PGA_2 methyl ester could also be prepared as a crystalline substance, mp 165.5–166°.

(9) (a) D. H. Nugteren, R. K. Beerthius, and D. A. van Dorp, *Recl. Trav. Chim. Pays-Bas*, **85**, 405 (1966); (b) M. Hamberg and B. Samuelsson, *J. Biol. Chem.*, **242**, 5336, 5344 (1967); (c) C. Pace-Asciak and L. S. Wolfe, *Biochim. Biophys. Acta*, **152**, 784 (1968); (d) C. Takeguchi, E. Kohno, and C. J. Sih, *Biochemistry*, **10**, 2372 (1971); (e) E. J. Christ and D. A. van Dorp, *Biochim. Biophys. Acta*, **270**, 537 (1972).

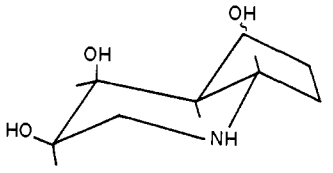
(10) See J. R. Vane, *Nature (London)*, **231**, 232 (1971).

(11) D. G. Ahern and D. T. Downing, *Biochim. Biophys. Acta*, **210**, 456 (1970).

with both ninhydrin and nitroprusside.[7] Significant retardation of **2** on borate-impregnated thin-layer systems and reaction of the compound with periodate were suggestive of a *vic*-glycol moiety in the molecule.[8]

Structure **2** was assigned on the basis of nmr spin decoupling.[9] The single proton H-4a (δ 2.05, m) is coupled to H-4 (δ 4.60, d of d, J = 3 Hz), to H-5 (δ 4.19, m, J = 6.5 Hz), and to H-7a (δ 3.05, m, J = 9 Hz). The secondary carbinol proton H-5 is coupled to a high-field proton (δ 2.27, m). H-7a is also coupled to one or more high-field protons at δ 1.71 (m). H-3 (δ 4.50, apparent d of d) is coupled to H-2_{ax} (δ 2.57, d of d, J = 7 Hz) and to H-2_{eq} (δ 3.23, d of d, J = 2 Hz); H-2_{ax} and H-2_{eq} are in turn coupled to each other (J = 12 Hz).

The fusion of the two rings is trans; H-4a and H-7a are both axial ($J_{4a,7a}$ = 9 Hz).[10] Since $J_{4,4a}$ = 3 Hz and H-4a is axial, H-4 is necessarily equatorial. Since H-3 is axial ($J_{2ax,3}$ = 7 Hz), the *vic*-glycol is cis ($J_{3,4}$ = 4.5 Hz). The configuration at C-5 could not be assigned with certainty from the coupling constants observed. From the available data, the configuration of **2** is either **3** (3*R*, 4*S*, 4a*R*, 7a*S*) or its enantiomer. The



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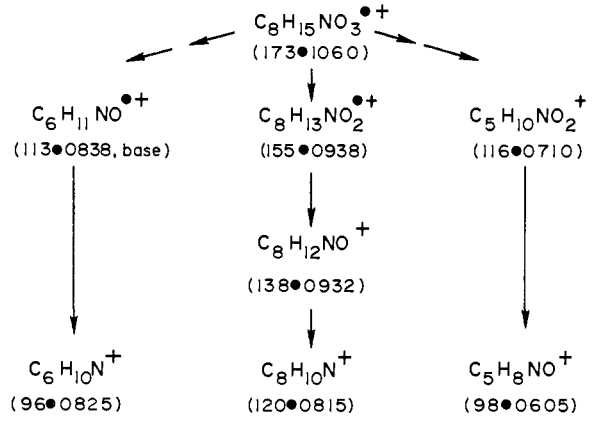
major fragments observed in the high-resolution mass spectrum of **2** (see Scheme I) are in accord with the assigned structure.

Scheme I



Acetylation[11] of **2** gave rise to the 1,3,5-triacetyl derivative in quantitative yield [*m/e* 299 (M), 239 (M − HOAc), 179 (M − 2HOAc), 137 (M − 2HOAc − C_2H_2O), 120; nmr δ 2.09 (9 H, s), 5.04 (H-4, d of d, $J_{3,4}$ = 4.5 Hz), 5.39 (H-5, m), and 5.70 (H-3, apparent d of d); ir 3420 (OH), 1740 (ester), and 1630 cm^{-1} (amide); no reaction with ninhydrin; one proton exchanged with D_2O (mass spectrometry)]. The remaining hydroxyl group (C-4) resisted acetylation (probably as a result of steric hindrance imposed upon this position by the adjacent acetoxy groups) and CrO_3–pyridine oxidation.[12]

Table I. Per Cent Incorporation of Precursors into **2**

Compd (sp act., μCi/μmol)	μmol added/ culture[a]	Incorpn, %[b]
DL-[6-^{14}C]Aminoadipic acid (11.7)	0.4	1.8
DL-[1-^{14}C]Lysine (2.7)	2.9	5.2
DL-[2-^{14}C]Lysine (0.02)	711.0	11.0
DL-[6-^{14}C]Lysine (2)	2.3	15.1
L-[U-^{14}C]Lysine (1.9)	3.2	5.9
DL-[4,5-^{3}H]Pipecolic acid (0.6)	45.8	3.2
DL-[*ring*-^{3}H]Pipecolic acid (250)	0.8	8.8
DL-[1-^{14}C]Pipecolic acid (0.006)	386.0	4.2

[a] Cultures, grown in 1-l. Roux flasks as previously described,[2] were drained at 12 days of age and resuspended in 50 ml of water containing the appropriate test compound. After 48 hr, mycelia were recovered and extracted with ethanol. [b] The product **2** was isolated by ion-exchange chromatography[6] and counted; % incorporation = (dpm of **2** recovered/dpm of compd added) × 100.

Preliminary experiments (Table I) indicate that the biosynthesis of **2** parallels that of slaframine.[2] The carbon skeleton of pipecolic acid appears to be incorporated *in toto;* the pathway 2-aminoadipic acid → L-lysine → pipecolic acid → **2** is probably operative. The origin of the remaining two carbons, C-5 and C-6, is unknown (DL-[1-^{14}C]- and DL-[3-^{14}C]serine, possible sources, were not incorporated). This biosynthetic route is at variance with those of other piperidine ring systems arising from poly-β-keto acids (coniine, conhydrine),[13] from dihydroxyphenylalanine (betanin),[14] and from isoprenoid precursors (as in the 2-pyrindine alkaloid skytanthine).[15]

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Resonance Raman Spectrum of the Matrix-Isolated Chlorine Molecular Anion Cl_2^-

Sir:

Argon matrices at 15–20°K have proven a fruitful means of trapping reactive intermediates, and recent[1]

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