small, it is likely that equilibrium between the spin states will be maintained under the usual experimental conditions.

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Studies on the Prostaglandin A_2 Synthetase Complex from *Plexaura homomalla*

Sir:

Recent research has established that the gorgonian *Plexaura homomalla* (*ex* Caribbean) is an unusually rich source (1.8% net weight) of prostaglandin A_2 (PGA_2) and its acetoxy methyl ester.[1-3] We were intrigued by the possibility that the prostaglandin synthetase of this easily available "sea whip" variety of coral might be highly active and/or abundant and, further, by the chance that the enzyme(s) might be solubilized, purified, and affixed to a column so as to allow an *in vitro* enzymic synthesis of prostaglandins in a continuous flow system. In addition it seemed that the study of the *Plexaura homomalla* synthetase complex might lead to worthwhile insights regarding the biosynthesis of prostaglandins. The following summarizes our endeavors to isolate and characterize this enzyme complex.

A small sample of *Plexaura homomalla* was collected *ca.* 0.5 mile north of McArthur's point, New Providence, Bahamas, and was immediately packed in Dry Ice to preserve enzymatic activity.[4] Examination of the indigenous prostaglandin isolated by extractions with ethyl acetate confirmed that the *Plexaura homomalla* in this area contain exclusively prostaglandins with the 15*S* (natural mammalian) configuration.[2]

We were disappointed to find that the incubation of tritiated arachidonic acid with a freshly prepared homogenate of *P. homomalla* in 0.05 *M* Tris or phosphate buffer at pH values from 6 to 8.5 and 28–29° did not result in the production of significant amounts of PGA_2, its methyl ester, or 15-*O*-acetyl derivatives. However, it was found that if the buffered homogenate were prepared using sea water, the enzymic synthesis of PGA_2 could be detected easily. It was further determined that the activity or lack of same of the PGA_2 synthetase depended simply on the presence of an appropriate concentration of sodium chloride.

Utilizing our observations that this prostaglandin synthetase has an optimum pH range of 7.5–8.0 and requires an optimum salt concentration of 1 *M*, we developed the following procedure to obtain active preparations of soluble PGA_2 synthetase. Frozen coral (*ca.* 2 g) was cut into small pieces and placed into a blender cup with 20 ml of ice-cold pH 8.0 buffer (0.05 *M* Tris and 1 *M* NaCl). The Waring Blendor was operated for 2.5 min on 70 V, and the resulting homogenate was centrifuged for 20 min at 8000*g*. The supernatant fraction was centrifuged at 100,000*g* for 90 min. The microsomal pellet contained the prostaglandin synthetase. Solubilization was effected by sonication of the pellet for 45 sec in 10 ml of pH 8.0 buffer (0.05 *M* Tris, 1 *M* NaCl) (0°) and the nadding sufficient glycerol and Triton X-100 to bring the final concentration of the latter to 5 and 0.5%, respectively.[5] This treatment did not enhance activity and did not stabilize the synthetase complex, since 80% of the activity was lost after standing 7 hr at 0°.

The incubation procedure was as follows. One milliliter of the above solution diluted 1:1 with buffer (pH 8.0, 0.05 *M* Tris, 1 *M* NaCl) was incubated with 25 μg of 2-tritioarachidonic acid for 20 min at 29°. After addition of 1 g of NaH_2PO_4 to quench the reaction, the solution was extracted five times with ethyl acetate. The residue, after removal of the solvent, was chromatographed on 20-cm long Brinkmann F-254 silica gel tlc plates using two developments with 2% acetic acid, 7% dioxane, and 91% benzene. Three major product bands were found: band I (10%) with an R_f of 0.4 was PGA_2; bands II (40%) and III (20%) (the identity of which are unknown) moved with R_f values of 0.52 and 0.62, respectively.[6]

Band I was confirmed to be PGA_2 by converting the extracted band to PGA_2 methyl ester using diazomethane and then to the 15-trimethylsilyl ether of PGA_2 methyl ester. In each case the labeled product displayed chromatographic behavior identical with the standard. Final confirmation was obtained by preparing the semicarbazone of band I, mixing it with the semicarbazone of authentic PGA_2,[7] and recrystallizing the mixture. Constant activity and constant melting point were obtained

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(4) If the coral is allowed to stand for 6 hr at 0°, the activity of the prostaglandin synthetase preparation diminishes by 60%.
(5) Solubilization was established, since the synthetase could not be precipitated by centrifuging at 100,000*g* for 30 min.
(6) Bands II and III do not seem to be derived from the postaglandin synthetase. The enzymes responsible for their production can be separated by either $(NH_4)_2SO_4$ precipitation or by column chromatography on agarose.
(7) We thank Drs. John Fried and Otto Halpern of Syntex Research for generous samples of PGA_2.

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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(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°.

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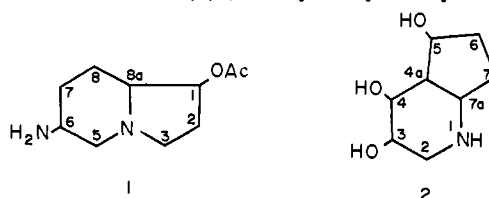
Isolation and Characterization of a 1-Pyrindine Fungal Alkaloid[1]

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-pyr-



indine). Apparently, this is the first example of a 1-pyrindine isolated from a biological source.

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

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