PREPARATION OF SUBSTITUTED PHENYL PYRROLES THROUGH THE METABOLISM OF TRYPTOPHAN ANALOGUES

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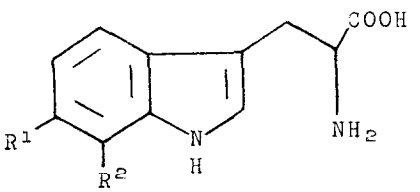
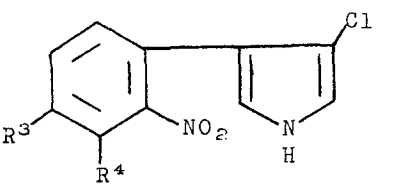
We have recently shown (Lively et al., 1967) by the use of C^{14}-labeled precursors that tryptophan, I, is a direct precursor of the antifungal substance pyrrolnitrin,[a] II. Pyrrolnitrin, 3-chloro-4-(2'-nitro-3'-chlorophenyl)-pyrrole (Imanaka et al., 1965a; Nakano et al., 1966), is produced by several species of pseudomonads (Imanaka et al., 1965b); in our laboratory it is available from Pseudomonas aureofaciens ATCC 15926. This organism when grown on a complex or synthetic medium is capable of converting either endogenous or exogenous tryptophan to pyrrolnitrin. Thus, the addition of 500 µg/ml of dl-tryptophan to the culture mentioned resulted in increasing the yield of pyrrolnitrin from 50 to 100 µg/ml of broth. For the multi-step process, this represents a minimum yield of 8%, based on exogenous dl-tryptophan.

We have now been able to add the tryptophan analogues, dl-6-fluorotryptophan (III) and dl-7-methyltryptophan (IV) to fermentations of P. aureofaciens and to recover the

[a] Pyrrolnitrin is available commercially in Japan from Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan, under the name PYROACE. It is used in the treatment of superficial fungal infections.

pyrrolnitrin derivatives, 3-chloro-4-(2'-nitro-3'-chloro-4'-fluorophenyl)-pyrrole[b] (V) and 3-chloro-4-(2'-nitro-3'-methylphenyl)-pyrrole[c] (VI), respectively. These new substances possess significant antifungal activity.





I $R^1 = R^2 = H$

III $R^1 = F$, $R^2 = H$

IV $R^1 = H$, $R^2 = CH_3$

II $R^3 = H$, $R^4 = Cl$

V $R^3 = F$, $R^4 = Cl$

VI $R^3 = H$, $R^4 = CH_3$

The media used and fermentation conditions are essentially those described for the production of pyrrolnitrin. The new metabolites are produced from the appropriate precursor when the organism is grown on either a complex (sucrose, molasses, corn steep, malt extract, and casein peptones) or chemically defined medium (glycerol and ammonium chloride as sole sources of carbon, nitrogen, and halogen). The tryptophan analogues were added to 60 ml of P. aureofaciens culture in 250-ml flasks which had been previously incubated for 24 hours at 30° on a rotary shaker. The fermentation was allowed to continue for 120 hours before harvesting the culture. Thin layer chromatography (silica gel, E. Merck AG. Darmstadt) in hexane-benzene (7:3),

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[b] 4'-Fluoropyrrolnitrin.

[c] 3'-Methyl-3'-dechloropyrrolnitrin.

developed, dried and redeveloped five times, clearly separated the new compounds from pyrrolnitrin as shown by the use of ultraviolet light or a H_2SO_4 spray.

4'-FLUOROPYRROLNITRIN (V). The addition of 300 µg/ml of dl-6-fluorotryptophan[d] to the culture growing as described by Lively (1967) led to the appearance of a new metabolite running slightly above pyrrolnitrin on thin layer plates (Table 1). After 120 hours, two volumes of methanol were added and the broth filtered. The filtrate was concentrated to 1/3 volume, adjusted to pH 10.5 and extracted with toluene and then chloroform. The majority of the pyrrolnitrin and new metabolite were extracted in the toluene; the chloroform extract contained about 10% of the new compound and was free from pyrrolnitrin. Both extracts were separately chromatographed on silica gel (Woelm, act. 1, ratio 100:1) in benzene, with the desired metabolites being eluted first and subsequently crystallized from hexane. NMR analysis (Table 1) indicated that the crystals from toluene, m.p. 146-148°, contained about 25% of pyrrolnitrin while those from the chloroform extraction were essentially pure 4'-fluoropyrrolnitrin, m.p. 155°C. Comparison data for pyrrolnitrin and the new metabolites are listed in Table 1. The molecular weight obtained from the mass spectrum was consistent with an empirical formula $C_{10}H_5FCl_2N_2O_2$, $M^+ = 274$ ($2Cl^{35}$). When assayed with a Neurospora species against a pyrrolnitrin standard (1000 µg/mg), the new fluoro-derivative

[d] dl-6-Fluorotryptophan was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin, No. 10,245-8.

assayed 1200 µg/mg. The fermentation broth assayed at 144 µg/ml which indicated a yield of 25% based on added dl-6-fluorotryptophan.

TABLE 1

PROPERTIES OF PYRROLNITRIN AND RELATED METABOLITES

	Pyrrolnitrin	4'-Fluoro-Pyrrolnitrin	3'-Methyl-3'-Dechloro-Pyrrolnitrin
M.P. °C	124.5	155	102-104
Rf[1] Values	0.36	0.41	0.30
Bioassay[2]	1000	1200	480
U.V. λ_{max}^{EtOH} mµ (a_m)	250 (7500)	248 (6550)	245 (qual.)
Mass Spectrum[3] M^+	256	274	236
I.R. $\nu_{max}^{CHCl_3}$ cm^{-1}			
>N-H	3450	3450	3450
$-NO_2$	1540	1550	1530
N.M.R. ($CDCl_3$) δ			
Pyrrole-H (A_2X)	6.77-6.82	6.78-6.85	6.8-6.81
AR-CH_3	---	---	2.38
AR-H	very tightly coupled ABC from 7.3-7.6	complex AB of ABX from 7.3-7.7	ABC pattern from 7.15-7.55

[1]System of hexane-benzene, 7:3, developed 5 times.
[2]Assayed against a Neurospora sp.
[3]Molecular ion containing only Cl^{35}.

3'-METHYL-3'-DECHLOROPYRROLNITRIN (VI). The fermentation and isolation were carried out as described above with 300 µg/ml of dl-7-methyltryptophan[e] being added to the fermentation broth. All of the antifungal metabolites were removed in the toluene extract which was purified by chromatography on silica gel in benzene-hexane (1:1). The crystalline methyl derivative had the properties shown in Table 1. The mass spectral molecular ion, $M^+ = 236$ (Cl^{35}), indicated the formula to be $C_{11}H_9ClN_2O_2$. The antifungal activity of the methyl analogue (VI) is 480 µg/mg.

The unusual utilization of exogenous tryptophans by P. aureofaciens to produce substituted phenyl pyrroles makes available a new group of antifungal substances which would be most difficult to obtain by conventional synthetic methods.

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[e]The dl-7-methyltryptophan was kindly prepared for us by Dr. E. C. Kornfeld and M. T. Suarez according to the method of H. N. Rydon, J. Chem. Soc., 705 (1948).