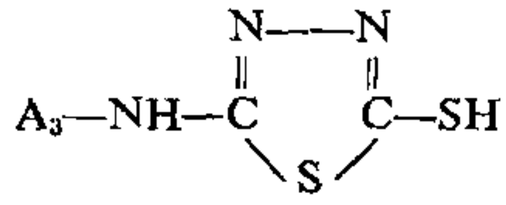
TABLE I



| Sl. No. | Ar. | Yield | M.P. °C | Mol. formula | % Sulphur Found | % Sulphur Reqd. | Dia. of zo. of inh. in mm |
|---|---|---|---|---|---|---|---|
| 1. | 4-Iodo-2-methylphenyl | 60 | 184 | $C_9H_8N_3S_2I_2$ | 18·46 | 18·34 | .. |
| 2. | 2, 5-Dimethoxyphenyl | 70 | 204 | $C_{10}H_{11}O_2N_3S_2$ | 23·66 | 23·79 | 10 |
| 3. | 2, 5-Dichlorophenyl | 65 | 130 | $C_8H_5N_3S_2Cl_2$ | 23·16 | 23·02 | 12 |
| 4. | 2-Bromo-4-methylphenyl | 68 | 186 | $C_9H_8N_3S_2Br$ | 21·32 | 21·19 | .. |
| 5. | 4-Chloro-2-methoxyphenyl | 62 | 172 | $C_9H_8N_3OS_2Cl$ | 23·58 | 23·40 | 11 |
| 6. | 4-Chloro-2-methylphenyl | 64 | 182 | $C_9H_8N_3S_2Cl$ | 24·72 | 24·85 | 10 |
| | Benzoic acid | | | | | | 13 |

by the method of Kazokov[5]. (ii) 2-*Arylamino*-5-*mercapto*-1, 3, 4-*thiadiazoles*: A mixture of appropriate 4-arylthiosemicarbazide (0·01 M), carbon disulphied (0·01 M) and dimethylformamide (20 ml) was refluxed on the water bath for about 1–1·5 hours at 65–70° C. The excess of the solvent was distilled off under reduced pressure. On cooling, the product separated out and was purified.

The details of the compounds thus prepared are listed in Table I.

*Pharmacology*: The prepared thiadiazoles were tested against five strains of fungi, *viz.*, *C. albicans*, *C. utilis*, *A. tenuis*, *A. niger* and *A. flavus* for fungicidal actvity at a concentration of 150 ± 10 μg/ml in 50% ethanol. The strength is reported by measuring the diameter of zone of inhibition of a particular microorganism (Table I) and the results were compared against beznoic acid.

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Department of Chemistry,
S.S.V. College
(Meerut University),
Hapur (U.P.),
*September* 4, 1975.

G. L. MAHESHWARI.
R. P. MAHESH
P. SINGH.

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## GENETICS AND BIOCHEMISTRY OF PYRIDOXINE REQUIRING MUTANTS OF *ASPERGILLUS NIDULANS*

IN our attempts to isolate a number of nutritionally deficient mutant strains of *Aspergillus nidulans* by ultraviolet irradiation of spores from a parent strain *y* ; $ribo_1$ (spore colour yellow and requiring riboflavin for growth), eight pyridoxine requiring mutants were obtained. Seven of them were found to be non-allelic by complementation tests[1]. The mutants were outcrossed with a strain *bi* ; $w_3$, $ade_3$ (white spore colour requiring both biotin and adenine for growth). Strains requiring only pyridoxine for growth were isolated from the perithecia and designated as $pyro_5$, $pyro_6$, $pyro_7$, $pyro_8$, $pyro_9$, $pyro_{10}$ and $pyro_{12}$, with the spore colours white, yellow which are recessive and the dominant green. Since all the mutant strains were available with white spore colours, excepting $pyro_8$, strains with white conidial colours were taken up for the present study, along with $pyro_8$ with yellow conidial colour.

The pyridoxineless mutants were allowed to grow at 23° C, 30° C, 37° C and 42° C on pyridoxine supplemented Minimal Medium[1] and were found to be temperature independent. The growth promoting effect of sugar phosphates, three carbon compounds of the glycolytic pathway, purines, pyrimidines, nicotinic acid and some other alleged precursors of pyridoxine are presented in Table I. From the results presented, it can be seen that β-glycerophosphate, dihydroxy acetone phosphate, 2- and 3-phosphoglycerates could promote the growth of mutant strain $w_3$ ; $pyro_9$. The mutant

TABLE I

*Effects of compounds related to pyridoxine or conceivably involved in its biosynthesis added in the absence of pyridoxine. Substances added at a level of 800 μg/10 ml of medium in 50 ml Erlenmeyer's flasks. Mycelial weights were obtained from 10 ml cultures after growth for 7 days. Results expressed in mg ± S.D.*

| Compounds tested | Strains tested | | | | | | |
|---|---|---|---|---|---|---|---|
| | $w_3$; $pyro_5$ | $w_3$; $pyro_6$ | $w_3$; $pyro_7$ | $y_1$; $pyro_8$ | $w_3$; $pyro_9$ | $w_3$; $pyro_{10}$ | $w_3$; $pyro_{12}$ |
| 1. Minimal Medium alone | — | — | — | — | — | — | — |
| 2. MM + all amino acids individually | — | — | — | — | — | — | — |
| 3. MM + Purines | — | — | — | — | — | — | — |
| 4. MM + Pyrimidines | — | — | — | — | — | — | — |
| 5. MM + Glucose-6-phosphate | — | — | — | — | — | — | — |
| 6. MM + Fructose-6-phosphate | — | — | — | — | — | — | — |
| 7. MM + Fructose-1, 6 diphosphate | — | — | — | — | — | — | — |
| 8. MM + β-glycerophosphate | — | — | — | — | 35·0±1·5 | — | — |
| 9. MM + Dihydroxy acetone phosphate | — | — | — | — | 32·0±1·5 | — | — |
| 10. MM + 3-Phosphoglyceric acid | — | — | 25·0±2·2 | — | 25·5±1·7 | — | — |
| 11. MM + 2-Phosphoglyceric acid | — | — | 29·5±3·0 | — | 33·0±1·4 | — | — |
| 12. MM + 3-Phosphoserine | — | — | 33·5±3·9 | — | — | 37·5±2·7 | — |
| 13. MM + Phosphoenolpyruvate | — | — | 35·0±2·5 | — | — | — | — |
| 14. MM + Pyruvate | — | — | 34·0±1·5 | — | — | — | — |
| 15. MM + Kynurenine | — | — | — | 33·5±1·7 | — | — | — |
| 16. MM + Formyl kynurenine | — | — | — | 34·0±1·8 | — | — | — |
| 17. MM + 3-hydroxy anthranilic acid | — | — | — | 38·0±1·6 | — | — | — |
| 18. MM + Nicotinic acid | — | — | — | 42·5±4·1 | — | — | — |
| 19. MM + Pyridoxine | 36·0±1·5 | 44·0±2·3 | 45·0±2·5 | 42·5±1·5 | 38·1±1·5 | 38·5±1·5 | 30·0±1· |

— Indicates inoculum weight too small to measure.

strain $w_3$; $pyro_7$ could grow in the presence of 2- and 3-phosphoglycerates, pyruvate and 3-phosphoserine. Only 3-phosphoserine could promote the growth of $w_3$; $pyro_{10}$. These results indicate that β-glycerophosphate, dihydroxy acetone phosphate, 2- and 3-phosphoglycerates, pyruvate and 3-phospho-

serine are growth promoting for some of the pyridoxine requiring mutants of *Aspergillus nidulans* and thus they may be involved in the biosynthesis of pyridoxine. It is significant to note that only the phosphoesters showed growth promoting activity, while glucose, fructose, glyceric acid, glycerol and serine did not promote growth for the respective mutant strains. This proves that the phosphoesters are active directly and not after hydrolysis (as could happen in the presence of phosphatase in culture filtrate).

Earlier observations made on a pyridoxine requiring mutant of *E. coli* by radioactive incorporation studies using isotopes of dihydroxy acetone phosphate and glyceraldehyde-3-phosphate[2,3,4 5] indicated that C-5, C-5′ and C-6 of pyridoxine could be derived from glyceraldehyde-3-phosphate and C–3, C–4 and C–4′ of pyridoxine from dihydroxy acetone phosphate, thus showing that the three carbon metabolic units in the glycolytic intermediates can serve as the source of cyclic precursors for the pyridoxine molecule. A series of studies made on a pyridoxine requiring mutant of *E. coli*[6,7,8] indicated that 3-phosphoserine could be a possible precursor of pyridoxine. These observations agree well with our observations.

One of the interesting observations made by us is that kynurenine, formyl kynurenine, 3-hydroxy anthranilic acid and nicotinic acid (which is a pyridine derivative like pyridoxine) are able to promote the growth of the mutant $y_1$ ; $pyro_8$. Neither amino acids, pyrimidines, purines nor sugar phosphates show any growth promoting activity in the place of pyridoxine.

The role of three carbon compounds and nicotinic acid in the biosynthesis of pyridoxine are being investigated in detail.

University T. M. VATSALA.
Biochemical K. RADHA SHANMUGASUNDARAM.
Laboratories, E. R. B. SHANMUGASUNDARAM.
A.C. College Buildings,
Madras 600 025, *June* 11, 1976.

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## *IN VITRO* TOXICITY OF CONSTITUENTS OF *RUMEX MARITIMUS* LINN. TO RINGWORM FUNGI

SEVERAL species of the genus *Rumex* Linn. (N.O. Polygonaceae) are used medicinally in Europe, South Africa and Madagascar, for various ailments including as an antidote to poisonous insect bites and cutaneous disorders[1]. In the screening of Indian plants for biological activity, alcoholic extract of *R. maritimus* Linn. was shown to exhibit activity *in vitro* against the two ringworm fungi, *Trichophyton mentagrophytes* and *Microsporum canis* for the first time[2] which prompted the present investigations.

*Isolation and identification of constituents.*—The alcoholic extract of the whole plant was fractionated into hexane and butanol-soluble fractions. The hexane-soluble residue was chromatographed over silica gel and three crystalline substances (A, B and C) were isolated. The butanol-soluble fraction on chromatography over silica gel yielded a crystalline substance D. *Substance A,* pale yellow flakes from chloroform-alcohol, m.p. 204°, $C_{16}H_{12}O_5$. A magenta colour with sulphuric acid and red-brown colour with ferric chloride indicated it to be an anthraquinone. $\nu_{max}^{(KBr)}$ : 3350, 1040 (OH), 1680 (free carbonyl), 1620 (chelated carbonyl) and 1560, 755 $cm^{-1}$ (aromatic). $\lambda_{max}$ : 226, 255, 268, 289 and 440 nm (log $\epsilon$ 4·43, 4·21, 4·24, 4·29 and 4·05). MS : m/e 284 (M+).

It formed a diacetate, m.p. 182–4°. $C_{20}H_{16}O_7$ (M+, 368), monomethylether, mp. 187–9°, $C_{17}H_{14}O_5$ (M+, 298), dimethylether, mp. 221–4°. $C_{18}H_{16}O_5$ (M+, 312) and was identified as physcion. *Substance B.* m.p. 137°, $(\alpha)_D$ — 35°. Monoacetate, mp. 126–7°. It was confirmed as $\beta$-sitosterol.

*Substance C,* yellow needles from chloroform-alcohol, m.p. 252–3°, $C_{15}H_{10}O_5$. It also gave a magenta colour with sulphuric acid and red-brown colour with ferric chloride. $\nu_{max}^{(KBr)}$: 3500, 1040, 1670, 1630, 1600, 765 and 730 $cm^{-1}$. $\lambda_{max}$ : 223, 253, 266, 289 and 439 nm (log $\epsilon$ 4·55, 4·26, 4·23, 4·29 and 4·10). MS : m/e 270 (M+).

It yielded a triacetate, m.p. 194–7°, $C_{21}H_{16}O_8$ (M+, 396). With diazomethane, it formed a monomethylether, mp. 203–4°, $C_{16}H_{12}O_5$ (M+, 284) which was identical with physcion. This established substance C as emodin.

*Substance D,* needles from methanol, mp. 280–4°. $(\alpha)_D$ — 44°, $C_{35}H_{60}O_6$. It gave positive Fiegel's test. Tetraacetate, m.p. 170°. On hydrolysis with 6 N HCl, it yielded $\beta$-sitosterol, and glucose which confirmed it as $\beta$-sitosterol-$\beta$-D-glucoside.

*Toxicity to ringworm fungi.*—Fragments of hyphae of the ringworm fungi, grown on Sabouraud's