

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.

1. Pontecorvo, G., Roper, J. A., Hemmons, L. M., MacDonald, K. D., and Bufton, A. W. J., *Adv. in Genetics*, 1953, 5, 141.
2. Hill, R. E. and Spenser, I. D., *Science*, 1970, 169, 773.
3. — and —, *J. Amer. Chem. Soc.*, 1971, 93, 518.
4. —, Rowell, F. J., Gupta, R. N. and Spenser, I. D., *J. Biol. Chem.*, 1972, 247, 1869.
5. — and Spenser, I. D., *Can. J. Biochem.*, 1973, 51, 1412.
6. Dempsey, W. B. *J. Bacteriol.*, 1969, 97, 1403.
7. — *Biochem. Biophys. Res. Commun.*, 1969, 37, 89.
8. — and Itoh, H., *J. Bacteriol.*, 1970, 104, 658.

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 disorders¹. 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 time² 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). λ_{max} : 226, 255, 268, 289 and 440 nm (log ϵ 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°, $(\alpha)_D - 35^\circ$. Monoacetate, mp. 126–7°. It was confirmed as β -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} . λ_{max} : 223, 253, 266, 289 and 439 nm (log ϵ 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°. $(\alpha)_D - 44^\circ$, $C_{37}H_{60}O_{11}$. It gave positive Fiegl's test. Tetraacetate, mp. 170°. On hydrolysis with 6 N HCl, it yielded β -sitosterol, and glucose which confirmed it as β -sitosterol- β -D-glucoside.

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

dextrose agar slants³ for fifteen days, were introduced into Erlenmeyer flasks containing 50 ml Sabouraud's dextrose broth to which 0.004% agar was added. The flasks were incubated at $28 \pm 1^\circ$ on shaker for 48 hr., the clear broth was dispensed in assay tubes and two fold serial dilution method was used for testing the activity. The test materials were dissolved in alcohol to obtain a 10 mg/ml solution. Suitable solvent and test fungi controls were maintained. Observations were made after 96 hr. incubation at $28 \pm 1^\circ$. The minimum inhibitory concentration (MIC) was calculated from the tube showing no visible growth. The effect of solvent in inhibiting the growth of test fungi was observed only in first two tubes and hence the plant material inhibiting the growth at a concentration of 250 $\mu\text{g/ml}$ (third tube) was considered active. The maximum concentration of the plant materials tested was 1000 $\mu\text{g/ml}$ and none of them altered the pH of the test broth (pH 5.8-6.0). The results are presented in Table I.

TABLE I
Antifungal activity of plant materials

Plant materials	MIC ($\mu\text{g/ml}$) against:	
	<i>T. menta-grophytes</i>	<i>M. canis</i>
Crude alcoholic extract	250.00	125.00
Hexane-soluble fraction	62.50	31.25
Butanol-soluble fraction	125.00	62.50
Aqueous fraction	Inactive	Inactive
Physcion	"	"
β -sitosterol and its glucoside	"	"
Emodin	250.00	125.00

R. maritimus Linn. has been reported to contain rutin, hyperin, rumarin, chrysophanol, physcion and emodin⁴. Emodic acid, aloë-emodin, rhein and rhein-like substances have been found only in fruits⁵. We have found only physcion, β -sitosterol, its glucoside and emodin in the Indian species investigated by us.

The crude alcoholic extract, hexane and butanol fractions and emodin from *R. maritimus* Linn. have been shown in this study to be toxic to ringworm fungi.

Thanks are due to Mr. Edward, Mr. Jagdish Kumar and Mr. S. K. Avasthi for technical assistance during this work.

Central Drug Research
Institute, Lucknow (U.P.),
December 23, 1975.

J. S. AGARWAL.
R. P. RASTOGI.
O. P. SRIVASTAVA.

1. Kirtikar, K. R. and Basu, B. D., *Indian Medicinal Plants*, Lalit Mohan Basu, Allahabad, 1933, 3.
2. Dhar, M. L., Dhar, M. M., Dhawan, B. N., Mehrotra, B. N., Srimal, R. C. and Tandon, J. S., *Indian J. exp. Biol.*, 1973, 11, 43.
3. Srivastava, O. P. and Gupta, R. N., *J. Sci. Industr. Res.*, 1958, 17 C, 87.
4. Bagrii, A. K., Kurmaz, B. V. and Litvinenko, V. I., *Khim. Prirodn. Soedin.*, 1966, 2, 85.
5. Fairbairn, J. W. and El-Muhtadi, F. J., *Phytochemistry*, 1972, 11, 263.
6. Walker, J. C., Morrel, S. and Foster, H. H., *Amer. J. Bot.*, 1937, 24, 536.

MANGO PEEL WASTE AS A SOURCE OF PECTIN

MANGO peel which constitutes 20-25% of the total weight of the fruit was suggested as cattle feed¹. However, no organised efforts have so far been made for its effective utilization. Pectin, a biological polymer containing partially methylated galacturonic acid is recognised as an indispensable ingredient in the food processing industry for the manufacture of jams, jellies and as a thickener in a variety of foods and in pharmaceutical preparations². An attempt was therefore made to extract pectin from mango peel and ascertain its physico-chemical properties. The characteristics of Alphonso mango peel pectin are shown in Table I

TABLE I
The physical and chemical characteristics of mango peel pectin (Mangifera indica, var. Alphonso)

Yield (d.w.b.) %	13.0
Moisture %	4.30
Ash %	0.55
Methoxyl content %	8.20
Anhydrouronic acid %	61.12
Degree of Esterification %	76.0
Molecular weight	105,000
Equivalent weight	964
Optical rotation (α) _D ²⁵	+182°
Setting time (mins)	10
Jelly grade	200

which indicate that this is comparable with good quality apple pectin suitable for commercial exploitation. The average yield of mango peel pectin on dry weight basis was found to be 13% as compared to apple pomace with 17% yield² and mandarin orange peel with 13.2% yield^{3,4}.