

from 2N HCl and analysed again by descending chromatography (Bu : Ac : H<sub>2</sub>O). Standard S-methyl and S-propyl cysteine sulfoxides and cycloallin were used as reference compounds.

#### Results and Discussion

The amino acids obtained from the ether extract (yield 0.1 g/Kg onion) on descending chromatography gave spots corresponding to S-methyl cysteine sulfoxide ( $R_f = 0.17$ ) and S-propyl cysteine sulfoxide ( $R_f = 0.35$ ). A third spot also appeared ( $R_f = 0.63$ ) which may be S-propyl cysteine<sup>1</sup>. The amino acids obtained from the ethanolic extract (yield 0.65 g/Kg onion) on chromatography gave spots corresponding to the above two sulfoxide amino acids and also cycloallin ( $R_f = 0.24$ ). The identity of the three sulfoxide amino acids in the above preparation was further proved by two-dimensional chromatography. About twelve unidentified amino acids also appeared on the same chromatogram. They may be the other onion amino acids reported in a similar study<sup>7</sup>. By recrystallisation a mixture of the above three sulfoxide amino acids was obtained from fraction 1 [eluent from IRA-93 (OH) yield 0.3 g/Kg onion] and pure cycloallin from fractions 2 and 3, yield 0.2 g/Kg onion.

Some of the onion sulfur compounds which, Bandyopadhyay *et al.*<sup>3</sup> separated from the ether extract may therefore belong to S-methyl and S-propyl cysteine sulfoxides and their degradation products reported elsewhere<sup>8</sup>. The therapeutic effects of the ether extract of onion and its sulphur compounds<sup>4,9-11</sup> and also the importance of its sulfoxide amino acids with relation to sulphur metabolism<sup>12</sup> warrant further investigation on this vegetable.

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### CHEMISTRY OF *TERMINALIA* SPECIES

#### Part XV\*. Chemical Examination of *T. procera* Roxb.

IN the course of our studies on the heartwoods of *Terminalia* species<sup>1-4</sup>, a sample of heartwood of *T. procera* Roxb. was secured from Andaman islands. Preliminary examination showed the presence of tannins and phenolic compounds, but no triterpenes.

The hexane extract contained  $\beta$ -sitosterol (m.p. 136–37°, acetate m.p. 125–26°), identified by comparison with an authentic specimen. The chloroform solubles contained three components which could be separated by column chromatography on silica gel. From the analysis and by comparison with the authentic samples, they were identified as (i) ellagic acid [m.p. 360°, blood red colouration with Greismeyer's reagent<sup>5</sup>,  $\nu_{\text{max}}^{\text{Nujol}}$ : 3570(m), 3490, 1730–1705 (br), 1625, 1585, 1540 cm<sup>-1</sup>,  $\lambda_{\text{max}}^{\text{EtOH}}$ : 255, 366, with NaOAc 256, 278, 355<sup>6</sup>] (ii) 3, 3'-di-O-methyl ellagic acid [yield 0.03%, m.p. 325–27°, blood red colouration with Greismeyer's reagent<sup>5</sup>,  $\nu_{\text{max}}^{\text{Nujol}}$ : 3460 (br), 1715, 1580, 1550 cm<sup>-1</sup>,  $\lambda_{\text{max}}^{\text{EtOH}}$ : 248 (log  $\epsilon$  4.75), 372 (log  $\epsilon$  4.15) unaffected with NaOAc or AlCl<sub>3</sub><sup>6</sup>] and (iii) 3, 3', 4-tri-O-methyl ellagic acid [yield 0.035%, m.p. 285–87°, green-brown-yellow colouration with Greismeyer's reagent<sup>5</sup>,  $\nu_{\text{max}}^{\text{Nujol}}$ : 3440 (br), 1720, 1580, 1550 cm<sup>-1</sup>,  $\lambda_{\text{max}}^{\text{EtOH}}$ : 249 (log  $\epsilon$  4.65), 370 (log  $\epsilon$  4.05) unaffected with NaOAc or AlCl<sub>3</sub><sup>6</sup>]. Their identification was further confirmed by the preparation of tetra-O-methyl ellagic acid (m.p. 340–42°) using K<sub>2</sub>CO<sub>3</sub> and dimethyl sulphate in acetone. Ellagic acid was obtained (yield 0.5%) from the acetone extracts of the heartwood.

Ellagic acid is a common constituent of a number of species of *Terminalia*. The 3,3'-di-O-methyl ellagic acid was obtained from *Euphorbia formosanum* Hey<sup>7</sup> and it occurs as a glucoside in the

heartwood of *T. paniculata* Roth<sup>8</sup>. The 3, 3', 4-tri-O-methyl ellagic acid was isolated from the bark and heartwood of *Eugenia mairi* A. Cunn<sup>9</sup>. The co-occurrence of these two methyl ethers has been found for the first time in *T. procera*.

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#### THE UTILITY OF MANGANESE IN LACTOSE MEDIUM TO DIFFERENTIATE RHIZOBIA FROM AGROBACTERIA

AGROBACTERIA are the most common bacterial contaminants which resemble rhizobia and are often confused with them during the routine isolation procedures. However, certain biochemical tests<sup>1,2</sup> have been proposed earlier to differentiate rhizobia from many species of *Agrobacterium*. In this connection, Clark<sup>3</sup> has reported that a modified Bergersen's medium<sup>4</sup> containing lactose and 20 m.e.  $Mn^{2+}/l$  could preferentially support the growth of all the species of *Agrobacterium* but exclude strains of clover and medic-rhizobia. In the present investigation, this finding has been verified extensively with tropical rhizobia of diverse origin comprising 115 isolates from *Cicer arietinum*, 12 from *Sesbania bispinosa* and *S. sesban*, and 8 isolates of *Agrobacterium* sp.; the latter was isolated as contaminants during routine isolation of nodule bacteria. All the isolates were inoculated on agar slopes of the following composition: lactose, 5.0 g;  $KNO_3$ ,

1.0 g;  $MgSO_4 \cdot 7H_2O$ , 0.1 g;  $Na_2HPO_4$  (anhydrous), 0.18 g; agar 12.0 g; FeEDTA (0.25% W/v), 10.0 ml;  $MnSO_4$  (33.5% W/v), 10.0 ml; pH, 6.8 and volume made upto 1000 ml by distilled  $H_2O$ . After incubating the cultures for 14 days, at  $28^\circ \pm 1^\circ C$ , observations were recorded for visible growth. Infectivity of all the isolates was tested on their homologous hosts according to the method of Wieringa and Bakhuis<sup>5</sup>.

It was interesting to note that all the isolates of rhizobia which nodulated their hosts could not also grow on the manganese lactose medium while the isolates of *Agrobacterium* showed luxuriant growth, thereby lending support to Clark's finding<sup>3</sup> and confirming the utility of the test as a diagnostic feature to differentiate *Rhizobium* from *Agrobacterium* (Table I).

TABLE I

Growth of rhizobia and agrobacteria on modified Bergersen's medium containing lactose and 20 m.e.  $Mn^{2+}/l$

Strains	Isolated from	No. of strains found positive	
		Growth on lactose-manganese medium	Nodulation on homologous host
<i>Cicer-rhizobia</i> (115)	<i>Cicer arietinum</i>	..	115
<i>Sesbania-rhizobia</i> (10)	<i>Sesbania bispinosa</i>	..	10
<i>Sesbania-rhizobia</i> (2)	<i>S. sesban</i>	..	2
<i>Agrobacterium</i> (5)	<i>C. arietinum</i>	5	..
<i>Agrobacterium</i> (3)	<i>S. bispinosa</i>	3	..

Figures in parenthesis are the number of isolates.

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