or phospholipids. Other components were free cholesterol, waxes, aliphatic alcohols and hydrocarbons.

2. This mixture was separated chromatographically on silica gel, the primary fractions being rechromatographed. Hydrocarbons, cholesterol esters and triglycerides, which were fairly well separated from each other, all showed subfractionation according to unsaturation. Esterified higher aliphatic alcohols were separated from free alcohols.

3. The fatty acids of the cholesterol esters were of an unusually high average unsaturation, owing mainly to a high content of C₂₀ and C₂₂ unsaturated acids and, to a less extent, to a rather low content of saturated acids.

4. The fatty acids of the triglycerides showed considerable resemblance to those of haddock-depot fat and, alone among the flesh lipids, contained a major proportion of hexadecenoic acid.

5. A triglyceride fraction, less strongly adsorbed on silica gel than the bulk of the triglycerides, contained unsaturated fatty acids much enriched in C₁₆ and C₁₈ derivatives at the expense of more highly unsaturated C₂₀ and C₂₂ acids, and hence its mean unsaturation was much lower.

6. Each class of lipid appears to have a characteristic fatty acid composition.

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Amino Acids, Fruit Acids and Polyols of Myrobalans

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Myrobalans are the dried fruit of an Indian tree, *Terminalia chebula* Retz, and they are used extensively for tanning. The ripe fruit is a hard yellow drupe, 3–5 cm. long, ovoid in shape, and containing a single seed, usually 2 cm. long and 1 cm. in diameter. When dry the fruit becomes five-ribbed.

There have recently been isolated from these dried fruit two crystalline ellagitannins, chebulagic acid (Schmidt & Nieswandt, 1950) and corilagin (Schmidt & Schmidt, 1952b), the constitutions of which (Schmidt & Nieswandt, 1950; Schmidt, Schmidt & Herok, 1954; Schmidt, Blinn & Lademann, 1952; Schmidt & Schmidt, 1952a),

together with that of a third tannin, chebulinic acid, have now been elucidated. Reference to two-dimensional chromatograms, Fig. 1 (cf. White, Kirby & Knowles, 1952), of the polyphenols and tannins in methanolic extracts of myrobalans (43–53% tannin, hide-powder test), specially collected in Ceylon and in the Bhimlipatam, Coimbatore, Jubbulpore and Kolhapur districts of India, showed that chebulagic and chebulinic acids and corilagin were major constituents, accompanied by varying proportions of the following products of their complete and incomplete hydrolysis: chebulic acid, 3:6-digalloylglucose, ellagic acid, gallic acid and β -glucogallin. Small quanti-

ties of chebulagic acid and corilagin were isolated by Schmidt's methods, and ellagic acid was identified spectroscopically (Hathway, 1956). Other *Terminalia* species including *T. bellirica*, *T. citrina* Roxb., *T. pallida* Brandis (25–35% tannin, hide-powder test) were examined, and a strictly similar pattern of polyphenols was found.

As the major polyphenolic constituents have been accounted for and as there was no information relating to the amino acids, fruit acids and sugars of myrobalans, the present study was undertaken, particularly as this is the first Combretaceous plant to be investigated in this way. It should, however, be stated at the outset that as the present work was carried out with dried-plant material, some of the substances now detected may not occur free in the ripe fruit, but they may have arisen during drying from rapid chemical changes, owing principally to enzyme action. This limitation applies particularly to the sugars and possibly to the amino acids, but it may not apply with so much force when considering the cyclohexane derivatives and tannins, which are of special interest to this project, although it cannot be altogether ignored in these cases. As *Terminalia* are copious producers of aromatic compounds, which are located in high concentration in the fruit, it was nevertheless considered possible that this investigation might reveal stable intermediates of aromatic biosynthesis. Fresh information concerning the identity of the non-volatile acids is relevant to tanning.

Fig. 1. Two-dimensional chromatogram of the polyphenols and tannins of myrobalans. The material was applied in the position marked $\odot$ and run first in 6% acetic acid containing 2% formic acid, followed by benzyl alcohol-*tert.*-butanol-*isopropanol*-water (3:1:1:1, v/v) mixture containing 1.8% formic acid. Spots located by FeCl_3 - $\text{K}_3\text{Fe}(\text{CN})_6$: 1, chebulagic acid; 2, chebulinic acid; 3, corilagin; 4, gallic acid; 5, ellagic acid; 6, chebulic acid; 7, 3:6-digalloylglucose; 8, β -glucogallin.

EXPERIMENTAL

General. Evaporations were carried out in N_2 under reduced pressure, $<35^\circ$. Micro-evaporations were effected (35°) in 1 ml. centrifuge tubes, when a regulated stream of N_2 was directed from an adjustable capillary on to the liquid surface. Paper chromatography was carried out at $23 \pm 2^\circ$ in all-glass apparatus. Chromatograms were dried at room temperature, unless otherwise stated. A Hanovia mercury arc fitted with a Wood's-glass filter was used to examine chromatograms for fluorescent zones.

Systematic separation of a 80% methanolic extract of myrobalans into fractions of chemically similar substances

Whole myrobalans, from *T. chebula* Retz, grown in the Jubbulpore district of the Madhya Pradesh, were split open, the kernels removed, and the remaining tissue was pulverized. A portion (20 g.) of ground meal was percolated with 80% (v/v) methanol (400 ml.) for 64 hr., when plant debris was removed by filtration through a sintered-glass funnel, pressed, and washed (5×50 ml.) with more 80% methanol. Partial evaporation of the combined filtrate and washings left a soln. (130 ml.), which was diluted to 300 ml. and continuously extracted for 2 days with ethyl acetate at $50^\circ/100$ mm. in a Schacherl apparatus. Residual ethyl acetate was removed from the aqueous phase, which was subsequently treated with a small excess of saturated lead acetate soln. (8 ml.). The supernatant was centrifuged off, and the sediment was emulsified with six successive amounts of 60 ml. of water; the supernatants were centrifuged off after each emulsification. Sugars could not be detected in the last supernatant when 1 ml. of soln. was treated with 2 ml. of a 0.15% soln. of anthrone in H_2SO_4 . Lead was removed from the combined supernatants by H_2S treatment, and H_2S was sucked from the filtrate at the pump.

The resulting soln. (750 ml.) was percolated (40 ml./hr.) through a column (bed-volume, 20 ml.) of Zeo-Karb 225 cation-exchange resin (1.95 mg. equiv./ml.) in the H^+ cycle, and the column was then washed free from acid and from sugar, as tested for by the anthrone reagent, with 60 ml. of water. The total eluate (850 ml.) was percolated (40 ml./hr.) through a column (bed-volume, 100 ml.) of De-Acidite G anion-exchange resin (1.5 mg. equiv./ml.) in the OH^- cycle, and the column was then washed free from sugar with 500 ml. of water. Evaporation of the eluate (pH 7) left a thin syrup, which was dried over P_2O_5 at $20^\circ/1$ mm. for 3 days. The neutral fraction (1.7 g.) constitutes 8.5% by wt. of undried myrobalans.

The Zeo-Karb 225 column was eluted with 5N- NH_3 soln. Eluate (35 ml.) was collected before NH_3 'break through', when the column was eluted with more 5N- NH_3 soln. (100 ml.), and washed free from NH_3 with 100 ml. of water. Evaporation left a slightly coloured residue, which was dried over P_2O_5 at $20^\circ/0.1$ mm. The basic fraction (250 mg.) constitutes 1.25% by wt. of undried myrobalans.

The De-Acidite G column was eluted with N-HCl (50 ml.), followed by 0.1N-HCl (approx. 600 ml.) until chloride 'break through'. When organic acids are displaced from anion-exchange resins, only small amounts of high pK value acids are left on the column when chloride 'breaks through'. Displacement with 0.1N-HCl was, therefore, continued only for a very short period, when the column was washed (500 ml.) free from acid. Evaporation left a clean residue,

which was dried over KOH at 20°/0.1 mm. The acidic fraction (1 g.) constitutes 5% by wt. of undried myrobalans.

Similar separation of 80% methanol extracts, derived from commercial myrobalans of unknown origin, gave comparable results.

Paper chromatography of the neutral fraction

An aqueous solution (0.2 ml.) of the neutral fraction (40 mg.) was spotted ($2 \times 2.5 \mu\text{l.}$) on start-lines, 4 cm. from the lower edge of Whatman filter papers nos. 1 and 2, 57 cm. in length. Single-way ascending chromatography was effected, with a *n*-propanol-ethyl acetate-water (7:1:2, by vol.) mixture (Albon & Gross, 1952) as solvent system. Multiple development (cf. Jeanes, Wise & Dimler, 1951) involving irrigations for 20 hr. gave a second solvent excursion of 35 cm., and an extended migration of all compounds. Similarly, when the same soln. was spotted on start-lines, 15 cm. from the upper edge of the filter papers, descending chromatography was effected by multiple development in *n*-propanol-ethyl acetate-water (8:1:1, v/v) (A. E. Flood, personal communication) and (7:1:2, v/v) solvent system. The papers were irrigated for 24 hr. with each mixture, solvent being allowed to drip from the serrated, bottom edges. To resolve glucose and galactose, the neutral fraction was also chromatographed (40 hr.) by the descending method, with the organic phase of the benzene-*n*-butanol-pyridine-water (1:5:3:3, by vol.) mixture as solvent system.

Absence of fluorescent zones on the unsprayed chromatograms confirmed the absence of fluorescent polyphenols from this fraction, and the remaining polyphenols were shown to be absent by treating a chromatogram with ferric chloride-ferricyanide reagent.

Spraying reagents were employed as follows: (a) Spraying with a *p*-anisidine hydrochloride (Hough, Jones & Wadman, 1950) solution in water-saturated *n*-butanol, followed by treatment (3–5 min.) at 100°, was used to detect all sugars. (b) Albon & Gross's (1950) 1-naphthol- H_3PO_4 reagent, followed by treatment (10 min.) at 90°, was employed for the ketoses. (c) Bradfield & Flood's (1950) mixture of a methanolic soln. (100 ml.) of bromocresol purple (0.04%) and H_3BO_3 (0.1%), and 1% $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ soln. (7.5 ml.) was employed for the detection of hexitols. (d) Treatment with the Trevelyan, Procter & Harrison (1950) AgNO_3 -NaOH spraying reagents, followed by fixation in 4% $\text{Na}_2\text{S}_2\text{O}_8$, revealed *cyclohexitols* and reducing sugars. Compounds in the neutral fraction were recognized by comparing their reactions and locations on paper with those of authentic compounds run on the same chromatogram. Marker spots ($2.5 \mu\text{l.}$) of 1% solns. of sugars were used.

When an aqueous soln. of the neutral fraction was spotted along the start-lines of another batch of filter papers which were chromatographed by multiple development, longitudinal strips were cut from each paper and sprayed in order to locate the sugars on the unsprayed papers, which were then cut into transverse strips (Dent, 1947) for elution with water. Each mono- and oligo-saccharide eluate was evaporated separately to single drops and spotted on to fresh filter papers, which were rechromatographed by multiple development.

In parallel experiments, oligosaccharide eluates were evaporated separately in bulb tubes (Flood, Hirst & Jones, 1948). The residues were heated at 105° with *N*-

H_2SO_4 in the sealed tubes for 3 hr., when they were centrifuged and opened. Barium carbonate was added in small amounts and stirred into the liquid with a micro stirring-rod, until the supernatant was no longer acid to Congo red. The tubes were centrifuged, and the supernatants evaporated separately to single drops, which were spotted on fresh filter papers and rechromatographed by multiple development. The corresponding monosaccharides were recognized by comparison with markers, and simultaneously authentic samples of the suspected oligosaccharides were chromatographed, eluted, hydrolysed and rechromatographed in precisely the same way.

Electrolytic desalting of the basic fraction

Desalting was effected by means of a circulating Hg cathode, and an anode compartment, through which acid is circulating, separated from the sample by means of an ion-permeable membrane (Consden, Martin & Gordon, 1947). In the present work an improved electrolytic desalter was employed, which involved a reversed flow of Hg, and is of such a size and implicated such flow rates that the temperature of the sample never exceeded 30°.

The water lift was operated so that the Hg cathode circulated at uniform speed, and the levelling tubes were adjusted to afford a stationary Hg surface, on to which a soln. (1 ml.) of a sample (55 mg.) of the basic fraction was introduced. Water was circulated through the condenser surrounding the electrolytic cell and 2% (v/v) H_2SO_4 was passed through the anode compartment at $\leq 100 \text{ ml./min.}$ When the apparatus was supplied with d.c. at 230 v, the current decreased in 10 min. from an initial value of 300 ma to a steady minimal value of 150 ma. The desalted sample (pH 7) was removed, evaporated to 0.5 ml., and stored at 0°.

Small-scale paper chromatography of the amino acids

A sample (2.5 $\mu\text{l.}$) of the desalted soln. was spotted at a distance of 2 cm. from both edges of the lower left-hand corner of Whatman no. 1 filter papers, 25.5 cm. square, and chromatographed by the ascending method with *tert*-butanol-water-formic acid (695:295:10, by vol.) mixture (Underwood & Rockland, 1954b) as first-way solvent system. The solvent was irrigated for 19 hr. to within 1 cm. of the upper edge of the paper. To remove accumulated impurities from the paper, which would otherwise cause a badly serrated solvent front with the second-way solvent system, the chromatograms were cut 15 mm. below the solvent front and parallel to the upper edge of the paper. Chromatograms were then developed in the other dimension by the ascending method with the second-way solvent system, consisting of phenol-water (775:215, w/w) mixture to which 35% NH_3 soln. (10.6 ml.) was added (Underwood & Rockland, 1954b). Complementary two-dimensional chromatograms also bearing 2.5 $\mu\text{l.}$ samples of the desalted soln. were similarly developed with water-saturated phenol containing 1% (v/v) of 35% NH_3 soln. as first-way solvent system, and *s*-collidine-2.4-lutidine (1:1) mixture saturated with water as the second-way solvent system (Dent, 1948). Both solvent fronts were allowed to reach the upper edge of the paper (17 hr.). After each solvent excursion, chromatograms were dried (1 hr.) at 60–70°, and the amino acids were identified by comparing their ninhydrin reactions (Underwood & Rockland, 1954a) and locations on paper with those

of authentic compounds run on the same chromatograms. Marker spots (2.5 μ l.) of 0.5% solns. of the amino acids were used.

Paper chromatography of the fruit acids

An aqueous soln. (1 ml.) of the acidic fraction (50 mg.) was spotted (5 μ l.) on start-lines, 4 cm. from the lower edge of Whatman filter papers nos. 1 and 2, 57 cm. in length. Single-way ascending chromatography was effected with the following solvent systems: benzyl alcohol-*tert*.-butanol-isopropanol-water (3:1:1:1, by vol.) mixture containing 1.8% (w/v) formic acid, (1); phenol-water (3:1, w/v) mixture containing 0.9% formic acid, (2) (Stark, Goodban & Owens, 1951); propanol-35% NH_3 soln.-water (6:3:1, by vol.) mixture (3) (Hanes & Isherwood, 1949). Papers were irrigated for 20–24 hr., and dried (1 hr.) at 60–70°.

Cations were completely removed by the exchanger, for chromatograms sprayed with adjusted indicators did not expose zones due to cations.

Spraying reagents were applied in the manner described in the following notes: (a) treatment with the Trevelyan *et al.* (1950) AgNO_3 -NaOH spraying reagents, followed by fixation in 4% $\text{Na}_2\text{S}_2\text{O}_3$, was used to detect the acids; those possessing 1:2-glycol groups gave black spots, and other acids gave white spots. (b) Successive spray treatments with a 2.6% (w/v) sodium metaperiodate ($\text{NaIO}_4 \cdot 3\text{H}_2\text{O}$) soln.; and an 83% ethanolic soln. (120 ml.) of sodium nitroprusside (0.5 g.) and piperazine (0.5 g.), followed by treatment (5 min.) at 100° (Cartwright & Roberts, 1955), were used to detect quinic acid and related substances (Cartwright, Roberts, Flood & Williams, 1955). (c) Hanes & Isherwood's (1949) mixture of 60% (w/w) HClO_4 (5 ml.), 4% (w/v) ammonium molybdate (25 ml.), N-HCl (10 ml.) and water (60 ml.) gave with free phosphate a yellow spot which turned green-yellow after heating for 7 min. at 85°. Free and bound phosphates afforded blue spots after further heating (2 min.) at 110° followed by exposure to H_2S . (d) When the chromatograms were developed in acidic solvent systems, the acids were exposed as yellow spots against a green background by means of a solution of bromocresol green at pH 5.5 (Lugg & Overell, 1947). (e) When the chromatograms were developed in the alkaline solvent system, the acids were revealed by Universal indicator (British Drug Houses Ltd.) adjusted to pH 9–10 (Long, Quayle & Stedman, 1951) as red spots, which faded fairly rapidly. (f) The occurrence of α -oxoacids was investigated with a 10% trichloroacetic acid soln. of *o*-phenylenediamine (Wieland & Fischer, 1949). The plant acids were distinguished by comparing their reactions and locations on paper with those of authentic compounds run on the same chromatograms. Marker spots (5 μ l.) of 0.8% soln. of authentic acids were used.

Chromatograms were also developed on acid-treated Whatman no. 1 filter papers. A 20 mg. sample of the acidic fraction was heated (bath temperature, 120°) with N-HCl (0.2 ml.) in a sealed tube for 2 hr., when the tube was centrifuged and opened, and the reaction mixture evaporated until free from HCl . An appropriate solution of the residue was chromatographed on filter paper, as above.

RESULTS

The present work represents a qualitative and exploratory study of the amino acids, plant acids and polyols of myrobalans.

After removal of polyphenols and tannins from an 80% (v/v) methanolic extract, the remaining constituents were divided into neutral, basic and acidic fractions by ion-exchange chromatography. The absence of C_3 acids in the acidic fraction showed that Lobry de Bruyn transformation and degradation of hexoses had not occurred on the weakly basic anion-exchanger.

Single-way chromatography of the neutral fraction by multiple development (Jeanes *et al.* 1951) in the solvent systems described in the Experimental section enabled the polyols to be identified (Table 1). These assignments were substantiated by the elution, isolation and rechromatography of single constituents, and by the elution and hydrolysis of individual oligosaccharides, followed by the isolation and rechromatography of the corresponding monosaccharides. Glucose and hexitol predominated in the mixture, sucrose (approx. 1%) and fructose (approx. 1%) were present in quantity, and there was a small amount (approx. 0.2%) of a disaccharide, with the same R_f values, hydrolysis products and reactions on paper as gentiobiose. The hexitol was probably sorbitol, because of its widespread incidence in fruiting trees, but R_f values do not exclude the presence of mannitol and dulcitol. The remaining sugars occurred in small quantity, and were identified chromatographically as arabinose, maltose, rhamnose and xylose.

The complex mixture of amino acids necessitated the application of two-dimensional paper chromatography for the identification of constituents. Good results were obtained on small-scale chromatograms. Alternative pairs of solvent systems were selected in order to resolve on one chromato-

Table 1. *Multiple development of the sugars and hexitol*

Solvent system A: *n*-propanol-ethyl acetate-water (7:1:2, v/v); solvent system B: *n*-propanol-ethyl acetate-water (8:1:1, v/v) followed by *n*-propanol-ethyl acetate-water (7:1:2, v/v). R_g is the excursion relative to that of glucose.

	Solvent systems			
	A (Ascending method)		B (Descending method)	
	R_f	R_g	Excursion (cm.)	R_g
Disaccharide	0.19	0.42	6.0	0.32
Maltose	0.25	0.56	8.5	0.46
Sucrose	0.33	0.73	12.0	0.65
Hexitol	0.43	0.95	17.6	0.95
Glucose	0.45	1.00	18.5	1.00
Fructose	0.50	1.11	22.0	1.19
Arabinose	0.52	1.16	23.3	1.26
Xylose	0.56	1.25	26.0	1.40
Rhamnose	0.67	1.50	34.0	1.84

gram those amino acids which were incompletely separated on the other. Thus, Underwood & Rockland's (1954*b*) solvent systems were used to resolve leucine and isoleucine, whereas Dent's (1948) solvent systems separated γ -aminobutyric acid, tryptophan and valine, as well as alanine and tyrosine. The chromatographic behaviour of the amino acids and their proximate proportions is shown (Table 2). An unidentified amino acid spot is possibly due either to 6-amino:2-hydroxyhexanoic acid or to methionine sulphone (Coulson, 1955).

Single-way chromatography in acidic and alkaline solvent systems enabled several non-volatile fruit acids to be identified (Table 3), when R_F values and the reactions on paper, described in the Experimental section, were compared with those of authentic compounds. The principal acids found were shikimic (approx. 2.5%), quinic (approx. 1.5%), the highly polar acid(s) (approx. 1%) and free phosphoric acid. Small quantities of

dihydroshikimic and succinic acids were also identified. Dihydroshikimic acid was separated from shikimic acid in the phenol-water-formic acid system.

The reactions of acid X (Table 3) imply a relationship with the cyclohexanecarboxylic acids, and, if so, the R_F values would suggest a dehydroshikimic acid. There is tentative agreement with the reported R_F values (Davis & Weiss, 1953) for 5-dehydroshikimic acid (Salamon & Davis, 1953).

A large spot was detected by indicators which had a lower R_F than quinic acid in acidic solvents, and a higher R_F value than quinic acid in the alkaline solvent system. Specific reagents seemed to divide the spot into zones (*A* and *B*). Reaction with molybdate indicates the presence of ester phosphate(s), in which case the stability to hydrochloric acid resembles that of phytic acid (Lindenfeld, 1934), and the highly polar acid may turn out to be Davis & Mingioli's (1953) compound Z_2 , a monophosphoshikimic acid.

Table 2. *Chromatographic behaviour of the amino acids and their proximate proportions*

Amino acids	R_F in solvent systems				Relative amounts of the amino acids found
	Underwood & Rockland (1954 <i>b</i>)		Dent (1948)		
	I	II	I	II	
Alanine	0.43	0.61	0.66	0.25	+
γ -Aminobutyric acid	0.53	0.78	0.83	0.19	+
Arginine	0.20	0.86	0.94	0.15	++ +
Asparagine	0.20	0.45	0.52	0.22	+++
Aspartic acid	0.32	0.17	0.20	0.27	++++
Cysteine	0.47	0.65	—	—	+
Glutamic acid	0.40	0.27	0.30	0.30	++
Glutamine	0.27	0.57	0.65	0.15	s.q.
Glycine	0.35	0.43	0.54	0.26	s.q.
Histidine	0.16	0.75	0.76	0.19	Trace
Isoleucine	0.81	0.81	0.87	0.49	s.q.
Phenylalanine	0.73	0.86	0.92	0.54	+
Proline	0.47	0.91	0.91	0.26	+++++
Serine	0.32	0.38	0.46	0.28	+
Threonine	0.36	0.53	0.57	0.33	+
Tryptophan	0.53	0.78	0.87	0.59	+
Tyrosine	0.43	0.61	0.71	0.58	+
Valine	0.64	0.78	0.83	0.35	+
(Unknown)	0.31	0.81	—	—	Trace

+ = 0.025%; s.q. = 0.01%.

Table 3. *Chromatographic behaviour of the fruit acids in acidic and alkaline solvent systems*

Acid	R_F in solvent systems			Colour reactions	
	1	2	3	AgNO ₃ -NaOH	NaIO ₄ -sodium nitroprusside
Succinic	0.73	0.70	0.25		
Unknown acid (X)	0.48	0.76	—	Brown	Pale yellow
Dihydroshikimic	0.40	0.70	0.45	Black	Greenish
Shikimic	0.40	0.56	0.44	Black	Green-yellow
Quinic	0.24	0.43	0.48	Black	Orange-yellow
Phosphoric	0.19	0.35	0	White	
{ Zone B	0.10	0.19	0.57	White	Pale yellow } Cream on standing
{ Zone A	0.05	0.14	0.60	Black	

The presence of ester phosphate and of amino acids in myrobalans recalls the early work of Phillips (1936) on this subject.

DISCUSSION

The substances detected in this investigation should now be considered in relation to their distribution in plants, especially amongst fruiting trees. The presence of fructose, glucose and sucrose was anticipated, but preponderance of glucose is characteristic of this dried fruit. Detection of small quantities of arabinose and xylose recalls the similar quantities of arabinose in the fig, and of xylose in the apple, apricot, cherry, peach, pear and strawberry (Aso & Matsudo, 1951; Ash & Reynolds, 1955; Genevois, Vitte & Guichard, 1955). The occurrence of free gentiobiose and rhamnose is rare, and they may therefore have arisen by hydrolysis of glycosides or polysaccharides when the fruits were dried, and this criticism may also apply to the other sugars detected.

The presence of a large quantity of hexitol is readily appreciated, for sorbitol is stored during the growth of the plum in place of hexoses, when the latter have attained a limiting concentration (Donen, 1939). In hexitol studies it has been shown that sorbitol occurs most frequently in the leaves and fruit of Rosaceae (Strain, 1937) and mannitol in Leguminosae (Plouvier, 1950), and when detached leaves of Rosaceae are floated on sorbitol solution they synthesize starch (Treboux, 1909), whereas leaves of Oleaceae similarly utilize mannitol. The absence of free galactose and ribose is usual in plant material (cf. Ash & Reynolds, 1955). The range of free amino acids found in the present study is typical of those detected in plant extracts in general, and it includes the ones which are present in the apple (Hulme & Arthington, 1950), citrus fruit (Underwood & Rockland, 1953) and the potato tuber (Dent, Stepka & Steward, 1947). It is possible that all eighteen amino acids occur free in intact plants, but some of them may, however, have arisen by hydrolysis of peptide-cell components when the fruits were dried. Quinic acid occurs widely in plants (Wehmer, Thies & Hadders, 1932), and its incidence has recently been authenticated in the apple (Hulme, 1951), apricot (Anet & Reynolds, 1955a), peach (Anet & Reynolds, 1953, 1955b) and plum (Dickinson & Gauler, 1954), as well as in grasses (Hulme & Richardson, 1954). In these cases, however, quinic acid is associated with the common fruit acids (citric, malic and tartaric acids) and chlorogenic acid. In this work, quinic acid occurs in the acidic fraction, whereas the common fruit acids and chlorogenic acid are absent. The acids of myrobalans accord with Hall's (1937) phytochemical relationship 'that

plants noted for their quinic acid content are not producers of citric acid', although this generalization has been found untrue in some other cases (Hulme & Richardson, 1954). Although the presence of shikimic acid in the fruit of *Illicium religiosum* and *I. anisatum* and in the leaves of *Ginkgo biloba* is well known, it has not previously been encountered concomitant with quinic acid, and the natural occurrence of dihydroshikimic acid has not been previously reported. Since the configuration of quinic and shikimic acids at carbon atoms C₍₃₎, C₍₄₎ and C₍₅₎ is identical (Dangschat & Fischer, 1950), the presence of the three structurally related acids in a single plant source is biochemically significant. There is also tentative evidence for the presence of a dehydroshikimic acid.

It is now relevant to refer briefly to microbial synthesis of aromatic compounds (aromatization). Davis (1951) showed that shikimic acid substitutes for the quintuple requirement of *Escherichia coli* and *Aerobacter aerogenes* mutants for *p*-amino- and *p*-hydroxy-benzoic acids, phenylalanine, tryptophan and tyrosine. 5-Dehydroquinic and 5-dehydroshikimic acids were normal intermediates in aromatic biosynthesis, but quinic acid was only utilized by means of a side reaction (Davis & Weiss, 1953) involving quinic dehydrogenase (Mitsuhashi & Davis, 1954). Eberhardt & Nord (1955) have shown that *Lentinus lepideus* produces *p*-hydroxyphenylpyruvic acid from glucose units. Heptulose was detected as an intermediate, and in consideration of Davis's work, Stevens & Nord (1955) believed that shikimic acid was also a precursor.

In view of Davis's bacteriological studies the presence of related cyclohexanecarboxylic acids, concomitant with polyphenols and tannins as shown in the present work, suggests that they may also be precursors of aromatic amino acids and polyphenols in the plant. Quinic, 5-dehydroquinic, 5-dehydroshikimic, shikimic and dihydroshikimic acids (I-V) are probably separated successively from each other by one-step enzymic reactions (Fig. 2). The work with bacteria and moulds suggests that the pathway leading to aromatic compounds leaves this reaction sequence at shikimic acid, but for plants the lead into the aromatic sequence remains to be established. Although quinic acid does not appear to be a metabolite in bacteria, its widespread incidence in plants suggests that in these it may be a metabolite. It is possible that the presence of chlorogenic acid depsides in other plants may have obscured the role of free cyclohexanecarboxylic acids as precursors in aromatization processes. In myrobalans, a primary product of aromatization is gallic acid (VI), two molecules of which differ in

Fig. 2. Scheme for aromatization in *Terminalia* species.

constitution from the remaining polyphenols, chebulic and ellagic acids, by the elements of hydrogen and water molecules. As already indicated in the Introduction, the tannins, chebulinic and chebulagic acids and corilagin are built up from these polyphenolic units. By studying the constituents and enzymic reactions of selected plants, it is intended to explore the course of aromatic biosynthesis leading to tannins.

SUMMARY

1. The examination of the polyphenols and tannins of the dried fruit of *Terminalia* species (myrobalans) by means of chromatographic methods is briefly summarized.

2. After removal of polyphenols and tannins from an 80% (v/v) aqueous methanolic extract of myrobalans, the remaining constituents are divided into neutral, basic and acidic fractions by ion-exchange chromatography, the amino acids iso-

lated after electrolytic desalting of the basic fraction, and paper chromatographic methods used for the provisional identification of constituents in the three fractions.

3. Glucose and hexitol (sorbitol) predominate in the neutral fraction, which also contains about 1% each of fructose and sucrose, a small quantity of gentiobiose and traces of arabinose, maltose, rhamnose and xylose.

4. The range of amino acids is typical of those detected in plant extracts in general, and it is possible that all eighteen of these substances occur free in intact plants.

5. In addition to small quantities of phosphoric and succinic acids, a family of related cyclohexanecarboxylic acids has been identified. These include quinic and shikimic acids as major constituents, together with smaller amounts of dihydro- and possibly dehydro-shikimic acids. Tentative evidence for the occurrence of a related ester phosphate is also presented.

6. Dihydroshikimic acid has not been previously detected in plant material.

7. The nature of the constituent amino acids, fruit acids and polyols is discussed against the background of authenticated plant products.

8. It is suggested that the structurally related cyclohexanecarboxylic acids may be formed by the plant as intermediates of aromatic biosynthesis, leading to the aromatic amino acids and the simple polyphenolic units from which the tannins of the *Terminalia* species are elaborated. It is intended to explore the course of aromatic biosynthesis leading to tannins in selected plants.

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