734. *The Tannins of Tara,* Caesalpinia spinosa (*Mol.*) *Kuntze.*

By D. F. Horler and H. E. Nursten.

A method has been devised for purifying the tannins of tara powder. Evidence has been adduced to show that one of the principal fractions of an ethanolic extract is a single substance with the constitution of a tetragalloylquinic acid. The two main fractions of an aqueous extract have also been investigated: each contains a digalloylquinic acid. Free quinic and shikimic acid are also present in tara powder.

The tara shrub is a legume native to the northern part of South America, where it is widely distributed. The tannin concentration is greatest in the pods, which are pale yellow and/or red and when crushed constitute the tara powder of commerce.

We have isolated from its ethanolic extract one of the main components of high molecular weight, designated A, as an amorphous solid. It is characterised by its specific rotation, its partition coefficient in two solvent systems, and an acetyl derivative with a definite melting point and specific rotation. Evidence of purity is provided by the close agreement of its counter-current distribution curve with a theoretical one and by paper chromatography. On complete hydrolysis it yields only gallic and quinic acid, the identification of the latter having been greatly facilitated by Haworth and Jones's [1] prior discovery of quinic acid as a degradation product of substances present in tara extracts.

[1] Haworth and Jones, personal communication, December, 1958.

Compound A is a tetragalloylquinic acid on the basis of its elementary analysis, its equivalent weight, its pK value of $<3{\cdot}6$, and estimation of the gallic acid content by two methods and of the gallic : quinic acid ratio (see Table 1). This constitution also accounts for the negative optical rotation [2] and the high tanning acidity [3] of tara extracts. Table 1 also gives data for some other fractions isolated (see Experimental section).

TABLE 1. *Analytical results for materials isolated.*

Substance:	P′	Q′	Q″	BC	A	A′	A″
Equiv. wt. (to pH 5·6)	392	—	—	—	855	829	—
C (%)	51·05	52·75, 52·45	52·35	51·65	52·3	52·6	52·4, 52·6
H (%)	4·0	4·35, 4·35	4·25	3·7	3·9	3·9	3·55, 3·5
Gallic acid, % determined:							
spectroscopically ...	60	72	72	77	—	79	82
direct method	53, 51	—	61, 61, 59	67, 67, 67	—	73, 76, 73	71, 73, 74
ratio	58, 57	77	69, 69, 69	75, 75, 75	—	77, 82, 80	76, 79, 81

	Calculated for galloylquinic acids				
	Monogalloyl	Digalloyl	Trigalloyl	Tetragalloyl	Pentagalloyl
Equiv. wt.	344	496	648	800	952
C (%)	48·8	50·8	51·9	52·5	52·9
H (%)	4·7	4·0	3·7	3·5	3·4
Gallic acid (%)	49·4	68·5	78·6	85·0	89·2

Similar, though not as extensive, evidence was obtained of the presence, in addition to the tetragalloylquinic acid, of trigalloyl- (BC), digalloyl- (P and Q), and monogalloylquinic acids. The identity of one component with theogallin, a monogalloylquinic acid first isolated by Cartwright and Roberts [4] from green tea, was proved by paper chromatography, confirming Haworth and Jones's work. Digalloylquinic acids predominate in aqueous extracts, whereas compounds of higher molecular weight are the main components of ethanolic extracts, the difference being attributed to the activity of enzymes.

Digallic and probably trigallic acid are present in partial hydrolysates of acid A, implying that this is a galloyl(trigalloyl)quinic acid, whereas the absence of digallic acid in the case of acids P and Q implies that they are bisgalloylquinic acids.

The polyphenols of tara powder comprise a family of closely related polygalloylacids, with a maximum number of galloyl groups per molecule greater than four. The constitution of the hydrolysable tannins known at present is based entirely on sugars acylated with gallic acid and other acids closely related to it, such as digallic, dehydrodigallic, hexahydroxydiphenic, chebulic, and brevifolincarboxylic acid. The tannins in which the place of the sugar is taken by quinic acid therefore provide a new sub-group. Direct evidence of the presence in one tanning material of substances containing different numbers of galloyl residues has not been previously available.

Free shikimic and quinic acid were detected chromatographically in the ethanolic extract. The only important tanning materials in which these acids had been identified previously are sumac [5] and myrobalans [6] and their presence led both Hathway [6] and Burton and Nursten [7] to postulate that gallic acid was formed in plants by Davis's shikimic acid route. The surprising fact about the tannins of tara is that quinic acid has taken the place of glucose and not that of a galloyl residue of the classical gallotannin molecule,

[2] Duff, in Harvey's " The Chemistry of Vegetable Tannins," Society of Leather Trades' Chemists, Croydon, 1956, p. 65.
[3] Ghosh, Thesis, Leeds University, 1956.
[4] Cartwright and Roberts, *J. Sci. Food Agric.*, 1954, **5**, 593; cf. Roberts and Myers, *ibid.*, 1958, **9**, 701.
[5] Catravas, Thesis, Leeds University, 1947; Catravas and Kirby, *J. Int. Soc. Leather Trades' Chemists*, 1948, **32**, 155; Henderson, personal communication, June, 1959; Haslam, personal communication, April, 1960.
[6] Hathway, *Biochem. J.*, 1956, **63**, 380; ref. 2, p. 99.
[7] Burton and Nursten, ref. 2, p. 57.

though the latter possibility follows more directly from the biogenetic scheme. Compounds of this type have yet to be found in Nature.

Experimental

M. p.s are corrected. Preparative evaporations were carried out under nitrogen. For carbon and hydrogen analyses we are indebted to Mr. F. R. Daubney. Analyses given in the Table on p. 3787 are not repeated; samples were dried at 100° *in vacuo*.

Paper Chromatography.—The ascending method, with Whatman No. 2 papers, 10″ square, in glass tanks kept in a room at 20° ± 1°, was used throughout. Three solvent systems were employed: (X) 2% v/v aqueous acetic acid; (Y) butan-1-ol–acetic acid–water (4 : 1 : 2·2 v/v), and (Z) benzyl alcohol–t-butyl alcohol–propan-1-ol–water–90% formic acid (75 : 25 : 25 : 25 : 3 v/v). Two-way chromatography in solvent X followed by Y was used throughout for phenolic materials.[8] Characterisation of quinic and shikimic acid was carried out one-dimensionally in solvents Y and Z.[6] Though separation usually improves, the R_F values in solvent Y decrease with time. The dry chromatograms were examined in ultraviolet light, mainly of 254 mμ (Hanovia " Chromatolite "), aromatic compounds appearing as dark areas against the fluorescence of the paper. For polyphenols, a spray containing both ferric and ferricyanide ions was found most effective, considerably exceeding ultraviolet light in sensitivity since it can detect about 0·01 μg. of gallic acid. Quinic and shikimic acid were detected by means of aqueous sodium metaperiodate, followed after 20 min. by ethanolic piperazine and then ethanolic sodium nitroprusside,[9] whereas spraying with 3% ethanolic aniline immediately after a buffered solution of sodium metaperiodate showed shikimic acid as a bright red spot,[10] and quinic acid gave a white area against the discoloured background. The nitroprusside spray is sensitive to about 0·1 μg. of quinic acid; the aniline spray is the more sensitive for shikimic acid. For sugars, butanolic aniline phthalate was used.[11]

Each spot on a chromatogram does not necessarily represent only a single substance and letters are used to refer to average *positions* on the X/Y chromatogram in terms of R_F values as follows:

	X	Y		X	Y		X	Y		X	Y
a	0·02	0·49	*f*.....	0·03	0·68	*l*.....	0·59	0·40	*s*.....	0·00	0·23
b.....	0·06	0·47	*g*	0·15	0·65	*m*	0·65	0·31	*t*.....	0·78	0·83
c.....	0·10	0·44	*h*	0·31	0·59	*n*	0·42	0·73	*v*	0·68	0·68
d.....	0·09	0·39	*j*.....	0·35	0·45	*p*	0·19	0·43	*w*	0·93	0·24
e.....	0·18	0·40	*k*	0·40	0·33	*q*	0·19	0·48			

Preparation of the Aqueous Extract of Tara Powder.—Tara powder (400 g.; moisture content 10·5%) was made into a slurry with an equal volume of acid-washed silver sand (1100 ml.) and poured into a wide glass tube. Next morning, water was passed through the mixture, pressure being applied to overcome the clogging due to swelling. On the fourth day, the eluate was colourless, 1600 ml. with a solids content of 250 g. having been collected. The bulk of this solution (1500 ml.) was evaporated to 870 ml. during 7 days at 30—40° and 770 ml. of the concentrate were continuously extracted at pH 6·5 for 3 days with ethyl acetate under a vacuum.[12]. The solution was then brought to pH 2·5 with phosphoric acid and continuously extracted with two successive lots of ethyl acetate under a vacuum during 4 days. The various ethyl acetate extracts were evaporated under a vacuum and the yields determined by drying to constant weight at 100°. These were: pH 6·5 extract, 0·8%; pH 2·5 extract I, 18·7%; and pH 2·5 extract II, 9·3%. The pH 6·5 extract, on chromatography, gave one main spot in position *h* and the presence of gallic acid was confirmed by Young's test (potassium cyanide). The two pH 2·5 extracts gave similar chromatograms, the major components being at *p* and *q*.

Preparation of the Ethanolic Extract of Tara Powder.—Tara powder (600 g.), in a slurry with 95% ethanol (1 l.), was poured into a glass tube as in the preceding experiment. Next morning, the extract was slowly run off, further 95% ethanol (3250 ml.) being added gradually at the top. The eluate was evaporated under reduced pressure at 30—40° and the residue (III) dried

[8] Roberts and Wood, *Biochem. J.*, 1953, **53**, 332.
[9] Cartwright and Roberts, *Chem. and Ind.*, 1955, 230.
[10] Yoshida and Hasegawa, *Arch. Biochem. Biophys.*, 1957, **70**, 377.
[11] Partridge, *Nature*, 1949, **164**, 443.
[12] Schmidt and Nieswandt, *Annalen*, 1950, **568**, 165.

in a vacuum-oven at room temperature to constant weight (351 g., 58%). 59% of material (III) is soluble in ethyl acetate.

A water extract of the residual tara powder gave spots on the chromatogram in positions *k*, *l*, *h*, *d*, and *c*, the last two being less marked. No spot at *p* or *q* was observed.

Purification of Material (III) *by Precipitation from Ethyl Acetate with Benzene.*—A solution of material (III) (200 g.) in water (300 ml.) was shaken with water-saturated ethyl acetate (500 ml.), and the organic layer was removed, and dried overnight (Na_2SO_4, 100 g.). The sodium sulphate was filtered off and washed with ethyl acetate (150 ml.). To the filtrate combined with the washings was added benzene (250 ml.) with stirring, precipitating a brown gum. The mother liquid was decanted and gave no further precipitate with benzene. The gum was dissolved in ethyl acetate (250 ml.) and reprecipitated with benzene (250 ml.), this process being repeated five times, at which point the precipitate was brown at first, followed by a large amount of white. The solid was next dissolved in ethyl acetate (250 ml.), and only sufficient benzene (160 ml.) was added to precipitate the brown substance. To the decanted liquor was added further benzene (340 ml.), and the resulting white material was collected. The process was repeated on the brown substance until no more white precipitate could be obtained from it (residue of brown substance, 6 g.). The white precipitates were combined (65 g.) and submitted again to the fractional precipitation, giving a material (IV) (33 g.), $[\alpha]_D^{23}$ −106° (in ethyl acetate, *c* 0·6), and 1 g. of brown material. Material (IV), on treatment with acetic anhydride and pyridine at room temperature, followed by precipitation in water and two recrystallisations from ethanol, gave colourless spherulites, m. p. 150—153°, $[\alpha]_D^{23}$ −70° (in ethyl acetate, *c* 0·6). After ten further recrystallisations, these had m. p. 148—152°, $[\alpha]_D^{23}$ −70·5° (in ethyl acetate, *c* 0·3). The acetylated material was insoluble in water or ether, but soluble in hot methanol, ethanol, or benzene, and in cold ethyl acetate, acetone, or chloroform.

Counter-current Distribution.—This was carried out in a manually operated, 50-tube machine, supplied by Quickfit and Quartz Ltd., and having a capacity for 25 ml. of each phase per tube.

Examination of Material (I + II) *in Ethyl Acetate–Water.*—This material (7·77 g.) was distributed over 50 tubes. The following fractions were obtained by combining appropriate tubes and evaporating the solutions under reduced pressure at 40°: tubes 3—13, 0·41 g.; 14—28, 2·08 g.; 29—45, 4·66 g.; 46—50, 0·27 g. Two-way chromatograms in solvents X/Y, after spraying, indicated polyphenols in the following positions for tubes 5, 23, 35, and 50, respectively: *m*, *l*, *j*, *k*; *p*; *h*, *q*; *a*, *g*. The materials in tubes 23 and 37 possessed specific rotations of −104 to −115° and −63 to −70°, respectively. All fractions gave a precipitate with the gelatin–salt reagent, but a positive Young's test was obtained only with the contents of tubes 29—45 (Q). These gave spots *h* and *q* on the chromatogram with about equal intensity. The solids obtained from tubes 14—28 were designated P.

The distribution of an aqueous extract of tara powder (without intermediate extraction by ethyl acetate) in ethyl acetate–water gave a picture similar to that given by (I + II), but complicated by excessive emulsification.

Purification of Material (I) *in Ethyl Acetate–Water.*—To a solution of material (I) (13·6 g.) in methyl acetate (200 ml.) was added benzene (154 ml.), the solution filtered, and further benzene (200 ml.) stirred in. A brown gum was deposited, collected, and dried to constant weight in a vacuum at room temperature (7·0 g.). Part (4·0 g.) of this was alternately dissolved in methyl acetate (50 ml.) and precipitated with benzene until a white powder (1·4 g.) was obtained. It then failed to give any evidence of gallic acid by Young's test. Part (0·74 g.) was distributed over 25 tubes in ethyl acetate–water, with the following results: tubes 8—13, 0·25 g.; 14—22, 0·34 g.; 23—25, 0·04 g. Specific optical rotations of the materials (*c* ~0·1) in the different phases of the tubes corresponding to the peaks in the distribution were: tube 11, lower phase −106°, upper phase −94°; tube 17, lower phase −135°, upper phase, −138°.

The contents of tubes 9—12 were evaporated under reduced pressure at room temperature to yield a pale brown powder (P′) (0·11 g.). The equivalent weight found by titration to pH 5·6 was 392, to pH 6·0 374 (Calc. for $C_{21}H_{20}O_{14}$: 496). Paper chromatography showed only one spot, in position *p*. A test with Young's reagent was negative, with the gelatin–salt reagent positive.

The contents of tubes 15—18 similarly gave a pale brown powder (Q′) (0·18 g.). Paper chromatography showed only one spot, in position *q*. A test with Young's reagent was negative, with the gelatin–salt reagent positive.

Examination of Material (III) *in Ethyl Acetate–Water.*—This material (3·85 g.) was distributed

over 50 tubes with the following result: tubes 2—6, 1·18 g.; 7—28, negligible; 29—45, 0·74 g.; 46—50, 1·18 g.

Examination of Material (IV) *in Ethyl Methyl Ketone–Benzene–Water.*—(*a*) This material (4·62 g.) was submitted to 50 operations of the apparatus, the phases from a 18 : 7 : 25 v/v mixture of ethyl methyl ketone–benzene–water being used. The results were: tubes 3—8, 0·32 g.; 9—18, 1·43 g.; 19—28, 1·53 g.; 29—47° 1·50 g. Material (0·48 g.) from tubes 22—28 was distributed over a further 50 tubes, with the same solvent system, the results obtained following very closely the theoretical values for a substance with a distribution coefficient of 0·90.[13] Tubes 18—32 then contained the main part (A) (0·40 g.). It had $[\alpha]_D^{24}$ −89° (in water, *c* 0·7) but no definite m. p. The equivalent weight found by titration to pH 5·6 was 855, to pH 6·0 770 (Calc. for $C_{35}H_{28}O_{22}$: equiv., 800). A paper chromatogram showed only one spot, in position *a*. Treatment with acetic anhydride and pyridine at room temperature gave a white solid, m. p. 147—152°, $[\alpha]_D^{22}$ −68° (in ethyl acetate, *c* 0·2).

(*b*) Material (IV) (5·39 g.) was distributed over 25 tubes with the same solvent system, results being: tubes 3—7, 1·01 g.; 8—15, 2·27 g.; 16—25, 1·03 g. The material from tubes 8—15 was distributed over a further 50 tubes in the same solvent system: tubes 3′—8′, 0·10 g.; 9′—18′, 0·59 g.; 19′—28′, 1·15 g.; 29′—47′, 0·58 g. The material in the upper phases of tubes 23′ and 25′ had $[\alpha]_D^{22}$ −94° (*c* 0·25). Both phases from tubes 20′—27′ were then combined and extracted four times with ethyl methyl ketone (100 ml.). Evaporation of the extract under reduced pressure at room temperature yielded a pale brown powder (A′) (0·58 g.), which gave a positive gelatin–salt test. The equivalent weight found by titration to pH 5·6 was 829, to pH 6·0 755. Chromatography gave only one spot, in position *a*. Acetylation as above, followed by three recrystallisations from ethanol, yielded an *acetate* as colourless spherulites, m. p. 149—152° (Found: C, 54·2, 52·8; H, 4·15, 3·55; Ac, 34·2. $C_{55}H_{48}O_{32}$ requires C, 54·1; H, 3·9; Ac, 35·2%), which showed diamond-shaped interference patterns under the polarising microscope; these were easily deformed by pressure on the cover slide but did not lose their shape on prolonged heating at 100°.

Examination of Material (III) *in Ethyl Methyl Ketone–Benzene–Water.*—Material (III) (4·71 g.) was distributed over 50 tubes with the mixture in the ratio 18 : 7 : 25 v/v, with the following results: tubes 3—8, 0·63 g.; 9—18, 0·99 g.; 19—28, 0·92 g.; 29—47, 0·81 g. The material from tubes 19—28 gave one spot, in position *a*, and a much weaker one at *h* on the chromatogram and had $[\alpha]_D^{23}$ −90° (in ethyl acetate, *c* 0·4). Since the material from tubes 9—18 gave as the main polyphenolic spot one in position *a*, either a considerable quantity of non-phenolic material must be present or there are at least two tannins which give a spot at this position.

Examination of Materials (I + II) *in Ethyl Methyl Ketone–Benzene–Water.*—A mixture of materials (I) (2·00 g.) and (II) (1·00 g.) was distributed over 25 tubes with the solvents in the ratio 18 : 7 : 25 v/v, with the following results: tubes 1—8, 1·91 g.; 9—15, 0·89 g. The material in tubes 1—8 gave spots on the chromatogram in positions *p* and *q*, whilst that from tubes 9—15 gave only one at *h*. The latter substance was negative to the gelatin–salt reagent, but strongly positive in Young's test.

Examination of Material (III′) *in Ethyl Methyl Ketone–Benzene–Water.*—White tannin (29 g.) was obtained from an aqueous solution of material (III) (155 g.) by extraction with ethyl acetate, counter-current distribution (contents of tubes 16—18), and repeated precipitation. Its specific optical rotation varied with concentration:

Concentration (%).....	3·56	1·78	1·19	0·594	0·356
$[\alpha_D^{21-22}$	−92·0°	−97·1°	−99·8°	−108·6°	−110·9°

A similar effect was observed by Catravas [5] with the dextrorotatory sumac tannin.

The tannin (4·26 g.) was distributed over 50 tubes with the same solvent system, with the following results: tubes 3—8, 0·62 g.; 9—18, 1·63 g.; 19—28, 1·17 g.; 29—47, 0·38 g. The upper phase of tube 14 had $[\alpha]_D^{21}$ −112°, the lower −134°. On paper chromatography, material from tube 4 showed spots in positions *j*, *l*, *p*, *q*, from tube 14 at *b*, *c*, and others in the same area, and from tube 24 one spot at *a* only. The contents of various tubes were combined and evaporated under a vacuum. Tubes 20—26 thus yielded a pale brown powder (A″) (0·72 g.), tubes 9—16 a pale brown powder (BC) (1·06 g.), and tubes 2—8 a pale brown powder (0·51 g.). A portion (0·49 g.) of the last material was distributed between ethyl acetate and water over

[13] Horler, Thesis, Leeds University, 1960.

50 tubes. Paper chromatography revealed spots in the following positions: tubes 2′—4′, *l*; tubes 8′—20′, *j*; tubes 14′—26′, *p*; tubes 30′—38′, *q* with possibly an adjacent spot. On evaporation to dryness in vacuum the yields obtained were: from tubes 2′—6′, 0·03 g., tubes 8′—24′, 0·16 g. (spots at *p*, *j*); tubes 26′—40′, 0·12 g. (Q″); tubes 42′—50′, 0·01 g.

Isolation of Gallic, Digallic, and Trigallic Acid by Means of Ethyl Methyl Ketone–Benzene–Water.—The mother-liquor obtained in the preceding experiment by precipitation with benzene from ethyl acetate was evaporated to dryness and part of the residue (0·85 g.) was distributed over 50 tubes. Paper chromatography revealed spots in the following positions, the weight recovered on evaporation being given in parentheses: tubes 13—27, *h* (0·57 g.); tubes 29—39, *g* (0·12 g.); tubes 40—43, *f* (0·015 g.). Tubes 38—40 gave spots at both *g* and *f*. The material from tubes 29—39 consisted of a pale brown powder, m. p. 276—278° (decomp.) (Found: C, 51·85; H, 3·35. Calc. for $C_{14}H_{10}O_9$: C, 52·2; H, 3·1%), not depressed on admixture with synthetic digallic acid. Both samples gave a spectrum with a maximum at 275 mμ (ε 18,700) and a shoulder at 285 mμ (ε 17,000).

These results are given in Table 2 together with those for distribution in ethyl acetate–water and partition coefficients found by direct determination.

TABLE 2.

	Ethyl methyl ketone–benzene–water		Ethyl acetate–water	
Position on the chromatogram	Partition coeff.	Tube containing max. amount *	Partition coeff.	Tube containing max. amount *
a	0·90	24	>4	50
f	—	40	—	50
g	—	34	>4	50
h	0·75	22	2·0	36
k, *l*, *m*	<0·17	<6	<0·2	<6
j	<0·17	<6	—	14
p	0·17	6	0·7	23
q	0·17	6	2·0	36

* By direct determination from 50-tube distribution.

Hydrolyses.—Hydrolysis, with N-sulphuric acid (10 ml.) under reflux, of materials A′ (25 mg.), P (87 mg.), and Q′ (28 mg.) was followed paper chromatographically, with results given in Table 3.

TABLE 3. *Position of spots obtained on hydrolysis.*

Time of hydrolysis (min.)	Substance A′	Substance P	Substance Q′
0	*a*	*p*	*q*
15	*a*, *h*	*p*, *h*, *l*	*q*, *h*, *d*, *k*
30	*a*, *h*	*p*, *h*, *l*	*q*, *h*, *d*, *k*
60	*a*, *h*, *g*, *d*, *c*, *b*	*p*, *h*, *l*, *m*	*q*, *h*, *d*, *k*
120	*a*, *h*, *g*, *d*, *c*, *b*, *e*, *f*, *j*, *k*, *l*, *m*	*h*, *l*, *m*	*q*, *h*, *d*, *k*
240	*a*, *h*, *g*, *d*, *c*, *e*, *f*, *k*, *l*, *m*	*h*	*h*

Spots *g* were shown to be due to *m*-digallic acid by co-chromatography with a synthetic specimen. Spot *f* is probably due to trigallic acid.[14] In each hydrolysis an arc appeared in position *w* but this was due to the action of the mineral acid, presumably on the paper itself.

Hydrolysis also often yielded spots in positions *s* and *x*, which gave a pale fluorescence in ultraviolet light and are probably caused by traces of ellagic acid (*s*) and a related compound, formed by oxidation. Occasionally, when the mixture had become overheated, spots in positions *t* and *v* were encountered; these were attributed to the formation of catechol and pyrogallol, respectively. During the hydrolysis of material Q′, weak spots at *l* amd *m* were formed transitorily and were thought to be due to the presence of a small amount of substance *p* in the material hydrolysed. During hydrolysis of material P a small spot appeared at *k*, probably due to substance *q*. No sugars were formed on hydrolysis. The presence of sulphuric acid interferes with the detection of quinic acid by spraying.

Chromatographic Identification of Quinic Acid.—Materials P, Q, and A′ (10 mg. each) were separately kept in N-hydrochloric acid (20 ml.) at ~100° for 20 hr. The solutions were then spotted on chromatography papers and run together with controls of quinic acid in N-hydrochloric acid in solvents Y and Z. In each case quinic acid was identified by its R_F values

[14] White, ref. 2, p. 7.

(0·36—0·40 in Y, 0·21—0·26 in Z) and its positive reaction to the metaperiodate–piperazine–nitroprusside spray. The R_F value of quinic acid in solvent Y is raised by the presence of mineral acid (0·34 to 0·38), there being a similar, but smaller, effect in Z. The R_F in Y, but not in Z, decreases progressively with the age of the solvent (0·40 to 0·27 in presence of mineral acid in a month).

Identification of Gallic Acid.—Apart from the chromatographic characterisation of gallic acid in the hydrolysates of all the fractions obtained from tara powder, material (IV) (2·99 g.) was treated with boiling N-hydrochloric acid (100 ml.) for 48 hr. The solution was then cooled and extracted with ether (10 × 100 ml.), the extracts being evaporated and the residue dried to constant weight at 100° (2·44 g., 81·5%). This gave an acetyl derivative, m. p. 171—172°, not depressed on admixture with authentic tri-*O*-acetylgallic acid.

Determination of the Ratio of Gallic to Quinic Acid Formed.—Hydrolysates were prepared by treatment of the fraction (A′ 25·0 mg.; Q′ 8·0 mg.; or P′ 13·0 mg.) in N-sulphuric acid (100 ml.) for 27 hr., cooling, and making up to 100 ml.

(*a*) Solutions of gallic acid monohydrate (twice recrystallised from water with charcoal) [Found: H_2O, 9·6%; equiv. (to pH 6·5), 187. Calc. for $C_6H_6O_5,H_2O$: H_2O, 9·6; equiv., 188] were prepared in 0·1N-sulphuric acid and their spectra determined on a Unicam S.P. 500 spectrophotometer. They exhibited a maximum near 270 mμ, where the absorbance was proportional to concentration at least up to 30 mg./l., with ε 8980 ± 350. Cartwright and Roberts [4] gave figures corresponding to ε 9095 in 50% aqueous ethanol, but Bradfield and Penney [15] recorded ε 7900 in ethanol. A sample of commercial gallic acid gave ε 7910. In water, in the absence of mineral acid, gallic acid has $\lambda_{max.}$ 265 mμ. An aqueous solution of quinic acid (90 mg./l.) showed no absorption at 270 mμ. In this work the absorbance at 270 mμ of the hydrolysates diluted ten times was used to calculate the percentage yields of gallic acid from our own value for ε. Results are in Table 1.

(*b*) Quinic acid remains unaffected by acid potassium permanganate solution below 30°, whereas the titre of gallic acid increases gradually with temperature from 20° to 100°. It is possible, therefore, to take known mixtures of the two compounds and to titrate them first in the cold (23° ± 1°) and then at 100°, readings being taken when the pink colour first persists for more than 30 sec. The ratio of the titres (V_{100}/V_{23}) was plotted against the ratio of the weights of quinic to gallic acid (Q/G) to give a straight line with the equation $V_{100}/V_{23} = 1{\cdot}28 + 1{\cdot}2Q/G$. The quotient V_{100}/V_{23} for a mixture of quinic and gallic acids (0·087 and 0·470 g.) remained unchanged after treatment in boiling 0·5N-sulphuric acid (50 ml.) for 5 hr. The ratio of the titres can therefore be used to estimate the ratios of quinic to gallic acid present in hydrolysates and the titre at 23° directly determines the amount of gallic acid (16·1 equiv. of permanganate absorbed). Aliquot parts of the hydrolysates (25 ml.) were titrated with 0·190N-permanganate. Results are in Table 1.

Chromatographic Identification of Quinic and Shikimic Acids in Material (III).—A solution of (III) (100 g.) in water (400 ml.) was extracted with 6 lots of ethyl methyl ketone (100 ml.) and the aqueous residue evaporated. The solid (20 g.) was dissolved in water (50 ml.) and passed through a column of Dowex 1-X10 anion-exchange resin (200—400 mesh; 10 g.) in the acetate form. Washing the column with water (600 ml.) brought through a polyphenolic fraction almost at its front. The eluate then obtained with N-acetic acid (200 ml.) was evaporated and the solid left (0·48 g., 0·3% on tara powder) chromatographed in solvents Y and Z. In each case, the metaperiodate–piperazine–nitroprusside spray produced two spots (R_F 0·49, 0·35; 0·40, 0·25, respectively), the more mobile one giving a bright red with the metaperiodate–aniline spray. The R_F values of authentic shikimic and quinic acids run in the same solvents at the same time were 0·48, 0·34, and 0·40, 0·25, respectively.

We thank Mr. H. Finch of D. W. McCarthy Ltd. for the supply of liberal quantities of tara powder, Dr. E. A. H. Roberts for samples of green tea, and Professor W. Bradley for use of a Hilger polarimeter. We are grateful for the award of a Procter Memorial Scholarship (to D. F. H.) and to Professor D. Burton, M.B.E., under whose general direction this work was carried out.

Department of Leather Industries,
The University, Leeds, 2.

[*Received, November 2nd,* 1960.]

[15] Bradfield and Penney, *J.*, 1948, 2251.