7. *The Catechins of Green Tea. Part I.*

By A. E. Bradfield, (Miss) M. Penney, and (in part) (Miss) W. B. Wright.

From the polyphenol fraction extracted from a green-tea infusion by ethyl acetate, *l*-gallocatechin and a *substance*, $C_{15}H_{14}O_7,H_2O$, probably *dl*-gallocatechin, have been isolated by partition chromatography.

Two flavonols, quercetin, present as a glycoside (Hlasiwetz and Malin, *Jahresber.*, 1867, 732; Deuss, *Rec. Trav. chim.*, 1923, **42**, 623), and kaempferol (Oshima and Ka, *Bull. Agric. Chem. Soc. Japan*, 1936, **12**, 133), have been isolated from green tea. Early work on the more characteristic polyphenols extractable by ethyl acetate from aqueous infusions of fresh green tea leaf or of green tea—the so-called " white tannin " of tea—indicated in addition to small amounts of gallic acid (Hlasiwetz, *Annalen*, 1867, **142**, 233) the presence of " condensed tannins," and occasionally small amounts of crystalline material were obtained (Deuss, *Meded. K. Proefstaat vor Thee*, 1914, **31**; Shaw, *United Planters' Assoc. S. India*, Bull. No. 4, 1935). More recently, by fractionation of the ethyl acetate extract of Japanese green tea, Tsujimura has isolated in low yield *l-epi*catechin (*Sci. Papers Inst. Phys. Chem. Res. Tokyo*, 1929, **10**, 253), *l*-gallocatechin (*ibid.*, 1934, **24**, 149), and 3-galloyl*epi*catechin (*ibid.*, 1930, **14**, 63; 1935, **26**, 186; 1931, **15**, 155). *epi*Catechin and gallocatechin have also been obtained from Formosan tea (Oshima, *Bull. Agric. Chem. Soc. Japan*, 1936, **12**, 103; Oshima and Goma, *J. Agric. Chem. Soc. Japan*, 1933, **9**, 948).

In all of six specimens of Java leaf examined by Deijs (*Rec. Trav. chim.*, 1939, **58**, 805), *epi*catechin and its 3-galloyl ester were present, but gallocatechin could only be obtained from three of the samples. In Ceylon leaf, gallocatechin has been reported (Lamb, *Tea Quart.*, 1938, **11**, 103). In addition to these crystalline substances,* all workers have encountered a considerable proportion of amorphous material, shown by Deijs (*loc. cit.*) to be a mixture and to contain combined gallic acid liberated in high yield by tannase, or less efficiently by acid hydrolysis. Harrison and Roberts (*Biochem. J.*, 1939, **33**, 1408) also find that prolonged hydrolysis of " tea tannin " from Indian tea yields gallic acid in some cases. On the other hand, Lamb and Sreerangachar (*Biochem. J.*, 1940, **34**, 1472) were unable to observe the liberation of gallic acid when *Aspergillus niger* was grown on a solution of an amorphous " tannin " from Ceylon leaf. In no case could sugars be detected as a product of either acid or enzymic hydrolysis. By reduction of the appropriate chalkone, following the method of Russell and Todd (*J.*, 1934, 1066), Oshima (*loc. cit.*) obtained an amorphous material, formulated as bis-(5 : 7 : 3′ : 4′ : 5′-pentahydroxy)flavpinacol, which he considered to be identical with an amorphous preparation obtained by him from Formosan tea. Apart from the difficulty of establishing the identity of amorphous powders, Russell's formulation of similar products as flavpinacols has been very strongly criticised (Freudenberg, Karimullah, and Steinbrunn, *Annalen*, 1935, **518**, 37).

In an attempt to determine more completely the composition of the polyphenol mixture present in an infusion of green tea, use has now been made of the method of partition chromatography on a silica gel column first introduced by Martin and Synge (*Biochem. J.*, 1941, **35**, 1358). Some 80% of the polyphenol extracted by ethyl acetate is soluble in wet ether, and by chromatographing this solution gallocatechin is readily separated in a yield of about 1·3% of the weight of the tea (8·5% of the total polyphenol), *i.e.*, about 4—5 times the yield obtained by Tsujimura's method. Corresponding to another well-marked band, a *substance* (*B*) is obtained (yield : 0·55% of the weight of tea, 3·6% of the total polyphenol), which separates from aqueous alcohol as a mixture of rhombs and minute needles in variable proportions, with no definite decomposition point, and has the composition $C_{15}H_{14}O_7,H_2O$. It yields an *acetyl* derivative $C_{27}H_{26}O_{13}$, m. p. 158·5—159·5°, isomeric with that of gallocatechin. Attempts to determine the molecular weight of this acetyl compound and to confirm the molecular weight of gallocatechin acetate by Rast's method were at first unsuccessful, owing to decomposition of the substances in the camphor solution. It was found that by raising the temperature fairly rapidly and making single observations on fresh specimens of the mixture, consistent results could be obtained, but although under these conditions no visible browning occurred, it was not felt that complete reliance could be placed on the calculated molecular weights. Recourse was therefore made to the *X*-ray method. The minute hair-like needles of the acetate of substance *B* were unsuitable for study, but for the molecular weight of the parent

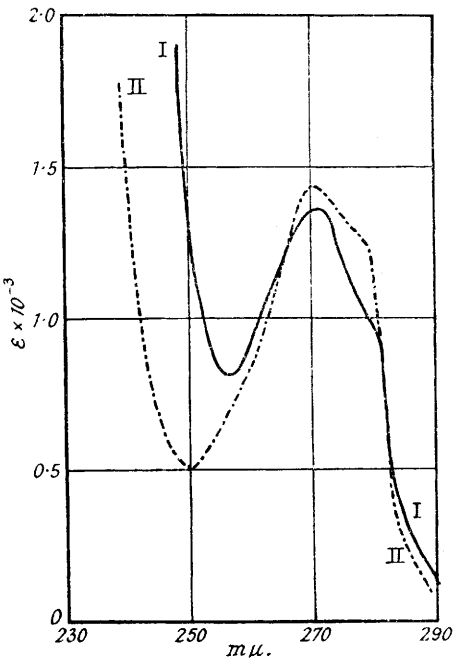
* Nierenstein (*Analyst*, 1936, **61**, 294) states that green Assam tea contains a crystalline substance, of which the crystalline methyl ether on hydrolysis yields 1 molecule of tetramethylacacatechin, 2 molecules of 3 : 4-dimethylgallic acid, and 1 molecule of trimethylgallic acid. No details are given.

substance a value of 324 ± 5 was obtained, in agreement with the value 324 calculated for $C_{15}H_{14}O_7,H_2O$. It is not yet possible to say whether this substance is a monohydrate or whether the water is present in the constitution and is eliminated in the formation of the acetyl derivative. A similar *X*-ray study of the crystals of gallocatechin acetate completely confirms the molecular weight of this substance.

The substance *B* is less soluble in water, alcohol and moist ether than gallocatechin, very sparingly soluble in dry ether, and insoluble in chloroform and hydrocarbons. It gives the pine-shaving test for phloroglucinol and, like gallocatechin, it gives with ferric ion in alcoholic solution an intense blue colour, fading slowly, and in aqueous solution a blue-black colour fading to colourless in a second or two; with sodium cyanide solution it gives a reddish-orange colour which fades and is restored by shaking the tube. The absorption spectra of the substance and its acetyl derivative (see fig.) are practically identical with those of gallocatechin and hexa-acetyl gallocatechin respectively. The substance is optically inactive in alcohol and in water–acetone (1 : 1) and is probably the *dl*-form of gallocatechin or its epimer. The investigation of this substance is being continued.

The remaining polyphenol fractions incompletely separated by the procedure now described may be resolved by a modification employing a different solvent. These fractions are under examination and their separation and properties will be described later. It may be mentioned that, as reported by other workers (Martin and Synge, *loc. cit.*; Gordon, Martin, and Synge, *Biochem. J.*, 1944, **38**, 65; Bell, *J.*, 1944, 473), some difficulty was at first experienced in obtaining results reproducible with different batches of silica. A satisfactory method of preparation of the gel is described in the Experimental part.

For purposes of identification, the optical characteristics, so far as they could be obtained, of the crystals of substance *B*, gallocatechin, and their respective acetates, and the interplanar spacings calculated from Debye–Scherrer photographs of the four substances are given below.



I. *Substance B.*
II. *Acetate of substance B.*

Experimental

(Microanalyses are by Drs. Weiler and Strauss, Oxford.)

Preparation of Silica Gel.—The volume (*n* c.c.) of concentrated hydrochloric acid required to neutralise the quantity of sodium silicate taken for the preparation described below was first determined by titration (methyl-orange) of the water-glass (*d* 1·42; 30 g.) diluted with water (500 c.c.). For the preparation, water-glass (210 g.) was diluted with water (300 c.c.) and concentrated hydrochloric acid (volume equal to 0·38*n*) run in from a burette in a continuous stream with vigorous stirring. Stirring and addition of acid were stopped immediately the solution thickened, and it rapidly set to a clear gel containing some opaque flecks, becoming in a few minutes entirely opaque. After being left for one hour from the time stirring was stopped, the stiff gel was broken up with a stirring rod, and during the next 35 minutes a volume of hydrochloric acid equal to 0·57*n* was added in 1-c.c. portions (total hydrochloric acid = 0·95*n*). The mixture was set aside for 3 hours, filtered through a Buchner funnel, and washed thoroughly without allowing the surface of the gel to crack. The gel was transferred to a beaker with water (200 c.c.) and after ageing for 24 hours, was filtered off, well washed, drained with suction for 30 minutes, and dried at 110—120°. The dried gel was stirred with concentrated hydrochloric acid (250 c.c.). After 30 minutes, water (250 c.c.) was added, and the gel was filtered off and well washed. Treatment with hydrochloric acid (150 c.c.) was repeated. After being filtered off and washed, the gel was stirred with boiling water (200 c.c.) for 10 minutes, cooled, filtered off, drained for 30 minutes, and dried at 110—120°. The product was ground and sifted to pass a 120-mesh (aperture 0·124 mm.) sieve and re-dried (yield ~ 50 g.). For the preparation of a gel with reproducible properties, attention is directed to the necessity of strict standardisation of the method of precipitation with acid, as well as of after-treatment.

Silica gel which has been used for chromatographic separation of tea polyphenols may be purified and used repeatedly. Solvent from material from the columns was removed in the steam-oven, the gel suspended in dilute sulphuric acid, and excess aqueous permanganate added. After 15 minutes the

suspension was decolorised with sulphur dioxide, filtered, and the gel washed. It was further treated once with hydrochloric acid, then with boiling water, dried, sifted, and re-dried as for new silica.

Chromatographic Procedure.—For the columns, a glass tube 3·15 cm. in internal diameter and 40—45 cm. long rested on a perforated nickel or silver disc, covered with filter-paper, in an adapter, the lower portion of which had a stopcock and was drawn out to a jet of about 2 mm. internal diameter. The upper portion of the glass tube was closed with a rubber bung carrying a 250-c.c. separating funnel, with the stem drawn out to a jet curved to touch the inside wall of the tube. Appropriate connections were also made for applying air pressure from an aspirator to the column. A thin cardboard scale was clamped behind the tube to facilitate the determination of the rate of flow of solvent. Silica gel (40 g.) was mixed with water (26 c.c.), suspended in wet, peroxide-free ether (200 c.c.), and the slurry poured into the tube, further small quantities of ether being required to complete the transfer. The silica was allowed to settle, wet ether flowing through the column without additional pressure. After ½ hr., air pressure from the aspirator was applied and adjusted to give the desired rate of flow (pressure 20—40″ of water). Occasional tapping assisted the column to settle down. When solvent had been flowing through the column under pressure for approximately 45 minutes, the space above the gel was filled to a depth of 15 cm. with ether, and the upper part of the gel lightly stirred to level the surface, and this quantity of ether allowed to flow through under pressure until the depth was about 1 mm. The polyphenol solution was then poured in through a funnel, made by drawing out a 1-cm. wide tube to a long capillary, held touching the inner wall of the tube. The solution and the first 50 c.c. of developing solvent (wet, peroxide-free ether) were allowed to flow through at a rate of 150 c.c./hr. (pressure 8—12″ of water). For the remainder of the development the rate of flow was increased to 350—400 c.c./hr. (pressure 20—30″ of water). Both during the settling down period and during working, patches occasionally appear where the gel has the appearance of drying locally. If neglected, these patches develop into cracks in the column. Local cooling by wrapping cotton wool moistened with ether round the outside of the tube (or the adapter) will cause these patches to disappear in 5—15 minutes, without interfering with the working of the column.

Under the conditions employed, the colourless bands were successively eluted and were detected by collecting four drops of the eluate in a test-tube every five minutes, and adding 2—3 c.c. of water and one drop of a *p*-nitrobenzenediazonium chloride solution (M/3), yellow to brownish-orange colours developing when polyphenol was present in the eluate. By timing additions of solvent to the column, the volumes of developing solvent corresponding to the appearance in the eluate of the beginning and the end of each band could be obtained from the times of the tests.

Extraction of Green-tea Polyphenols.—Distilled water (250 c.c.) and Ceylon tea (10 g.) were brought to the boil, maintained at the boiling point for 5—6 minutes, cooled, and filtered through muslin. The infusion (200 c.c.) and chloroform (150 c.c.) were refluxed for 15 minutes, cooled, and transferred to a separating funnel by pouring through a thistle-funnel reaching to the bottom (to avoid emulsification). After separation of the layers, extraction was completed in a continuous-extraction apparatus for 6—8 hours. Chloroform removes caffeine, some chlorophyll degradation products, and traces of carotenoid pigments from the infusion. The aqueous solution was separated, boiled for a few minutes while a current of carbon dioxide was passed through it to remove chloroform, and continuously extracted with ethyl acetate (12 hours). From the ethyl acetate extract the solvent was distilled off in a current of carbon dioxide, the latter portion under diminished pressure. The residue was washed into a dish with acetone, which was evaporated on the water-bath. The resinous residue broke up to a light brownish-orange amorphous powder (1·25 g.).

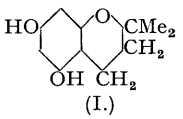
Chromatogram of Ether-soluble Polyphenols.—The orange powder obtained as above (2·0 g.) was rubbed with successive portions of wet ether (total volume 40 c.c.), and the solution, decanted from the insoluble portion (0·4 g.), chromatographed as described above. A barely visible pale green band passed rapidly down the column and appeared as a green solution in the eluate. It was rapidly followed by several incompletely separated polyphenol bands, the whole eluate from approximately 60 c.c. to 600 c.c. being collected as fraction (*a*). After a clear space, a well-defined band was collected as fraction (*b*), and a following band as fraction (*c*) (corresponding to the passage of approximately 700 to 1000 c.c. and 1200 to 1800 c.c. of solvent, respectively). During development, faintly coloured bands moved very slowly down the column. At the end, a narrow pink band was just visible 2—3 cm. from the top. Somewhat lower was a broad, faintly yellow band. These were not investigated. Fraction (*a*) was dried ($MgSO_4$), concentrated by distillation in a current of carbon dioxide, transferred to a dish, evaporated to dryness in a carbon dioxide stream at room temperature, and kept for later examination (1·07 g.). Fraction (*b*): The combined solutions from two columns were dried ($MgSO_4$) and concentrated by distillation in carbon dioxide to 30 c.c. A portion of the material crystallised out on standing. The remainder was obtained after rechromatographing the decanted solution on 40 g. of silica mixed with 26 c.c. of water. The eluate containing the required fraction was dried and evaporated to dryness as described for fraction (*a*); yield 0·14 g. from 4 g. of orange powder. Fraction (*c*) was dried, concentrated to 30 c.c., and rechromatographed from 40 g. of silica. When the band appeared in the eluate the next 150 c.c. of the eluate were discarded before the main portion was collected. Solvent was removed as described above. Yield 0·17 g. from 2 g. of the orange powder.

Substance B.—The fraction (*b*) (0·1 g.) was dissolved in water–alcohol (2 : 1 by vol.; 3 c.c.) at 70°, and the *substance* separated on cooling as a mixture of white rhombs and minute needles, the former usually predominating. Conditions were not found for obtaining either form exclusively. After drying over sulphuric acid, a specimen consisting mainly of needles decomposed in the neighbourhood of 160°, whereas a largely rhombic specimen decomposed at about 195°. In all specimens the decomposition point was far from sharp. No further loss in weight occurred on drying in a high vacuum at room temperature. At higher temperatures decomposition ensued; $[\alpha]_D^{20°} \pm 0·0°$ ($c = 0·37$, in alcohol; 0·42, in water–alcohol, 1 : 1 by vol.) (Found: C, 55·9; H, 5·08. $C_{15}H_{14}O_7,H_2O$ requires C, 55·6; H, 4·97%). Its *acetyl* derivative crystallised from dilute acetic acid in minute needles, m. p. 158·5—159·5°, $[\alpha]_D^{20°} \pm 0°$ ($c = 0·41$, in acetone) [Found: C, 57·8; H, 4·79; *M* (Rast), 575. $C_{27}H_{26}O_{13}$ requires C, 58·1; H, 4·69%; *M*, 558].

Gallocatechin (*Substance C*).—Fraction (*c*) (0·5 g.) was dissolved in water at 70° (3 c.c.), and the substance *C* crystallised in minute white needles, decomp. 217—218°, $[\alpha]_D^{22°}$ − 60° (*c* = 0·28, in alcohol) (Found: C, 58·8; H, 4·87%). It yielded an acetate as rhombic plates, m. p. 190·5—192°, $[\alpha]_D^{21°}$ − 14° (*c* = 0·51, in acetone) (Found: C, 58·1; H, 4·83%). These properties agree with the literature values for gallocatechin. For the centrifugal filtration of small quantities of gallocatechin and of the substance *B*, both of which rather readily become brown when exposed to air and light, especially when moist, a form of Skau tube (Fieser, " Experiments in Organic Chemistry," New York, 1941) with a sintered-glass filter disc (No. 2) was found advantageous.

Apparent Partition Coefficients.—It being assumed that in the chromatographic experiments the region of maximum concentration of polyphenol occurs midway in the band, the volume of solvent required to move this region the whole length of the column is $(v_1 + v_2 + v_s)/2 = V_m$, where $v_1 + v_2$ are the volumes of solvent which have passed through the column when the beginning and end respectively of the band are detected in the eluate, and v_s is the volume of the solution. Then *R* as defined by Martin and Synge (*loc. cit.*) is equal to la/V_m, where *l* is the length of the column and *a* is the cross-sectional area of the tube. Hence values for the partition coefficients between water and ether may be calculated by the equation of Martin and Synge. The mean values obtained were 29 for the substance *B* and 56 for gallocatechin. The calculation assumes that no adsorption takes place, a condition which may not be satisfied with silica prepared as described.

Absorption Spectra.—The absorption curve for substance *B* in alcohol (see fig.) showed a maximum at λ max. 271 mμ, with ε max. 1290, and its acetate a similar maximum at λ max. 271 mμ, with ε max. 1430. The spectra of gallocatechin and its acetate in alcohol are almost identical with the above two curves, with λ max. 271 mμ, ε max. 1340 and λ max. 271mμ, ε max. 1260, respectively. In all four curves a shoulder appears at about 280 mμ, but a definite secondary maximum for gallocatechin at this point, reported by Oshima (*loc. cit.*), was not observed. It is noteworthy that, as a fairly good approximation, the absorption maxima both for substance *B* and for gallocatechin can be represented as the sum of the maxima for the chroman (I) and for pyrogallol which have λ max. 272 mμ, ε max. 624 (Morton and Sawires, *J.*, 1940, 1052), and λ max. 266 mμ, ε max. 800 (Goslawski and Marchlewski, *Bull. Acad. Polonaise*, 1931, *A*, 383), respectively.



(I.)

By (Miss) W. B. Wright

Microscopical Examination of Crystals.—(a) *Substance B.* Substance *B* was obtained as prismatic crystals and as fibrous needles, the latter being too small for examination. The former had a mean refractive index of 1·62 and showed inclined and straight extinctions in elongated sections. Maximum extinction angle ~ 18°. *X*-Ray evidence established that the crystals belonged to the monoclinic system, with elongation along the *c* axis. The three principal refractive indices are ~ 1·58 for vibrations parallel to the diad axis, ~ 1·73 for those in a direction 15° from the *c* axis in the acute angle β, and ~ 1·58. For light travelling perpendicular to the *c* axis the crystals have a high double refraction ~ 0·12. Because of the smallness of the crystals an optic picture could not be obtained.

(b) *Acetyl derivative of substance B.* The crystals were fibrous needles of cross section ~ 1 μ. The mean refractive index was with difficulty found to be between 1·54 and 1·58. The fibres exhibited almost straight extinction. No further tests could be applied because of the small crystal size.

(c) *Gallocatechin.* The compound crystallised in bundles of fibrous needles of cross section <1 μ. The mean refractive index was determined with great difficulty and found to be *ca.* 1·54. The extinction direction was almost parallel to the length of the fibres. No further information could be obtained because of the small crystal size.

(d) *Gallocatechin hexa-acetate.* The crystals were colourless, foliated, and pinacoidal in form, the pinacoid faces being parallelograms with edge angles of approximately 65° and 115°. The extinction directions were parallel to the bisectors of the angles of the pinacoid faces, and the mean refractive index was 1·56. From *X*-ray evidence it was established that the crystals belonged to the monoclinic system, the pinacoidal faces being (001) faces, and the diad or ortho-axis lying parallel to the bisectors of the obtuse angles of these faces. Owing to the extreme thinness of the crystals (~ 13 μ) only the refractive indices parallel to two of the principal vibration directions could be evaluated, *viz.*, ~ 1·54 for vibrations parallel to the diad axis, and ~ 1·57 for those parallel to the [illegible] or clino-axis. The crystals possessed a high double refraction ~ 0·20 for light travelling perpendicular to the plane of the plates. An optic picture could not be obtained.

Debye–Scherrer Photographs.—Photographs of powdered samples of the four crystalline materials were taken on a flat plate camera, with a specimen-to-film distance of 5 cm. Copper-*Kα*, nickel-filtered radiation from an *X*-ray tube operated at 30 kv. and 30 ma. was used, and the specimen was rotated during the exposure. The exposure times varied from 5 to 6 hours. The diameters of the diffraction rings so obtained were measured, and the interplanar spacings, in *kX* units, calculated from these, are listed below. Relative intensities, determined visually, are shown in parentheses (V.S. = very strong, S = Strong, F.S. = fairly strong, M = medium, W = weak, V.W. = very weak). Owing to the complexity of the diffraction patterns, these spectra may not be complete.

Compound B.—(*Prismatic form*).

7·37 (F.S.)	6·66 (W.)	6·09 (F.S.)	5·78 (V.W.)	5·35 (F.S.)	5·09 (W.)
4·73 (F.S.)	4·27 (M.)	4·09 (M.)	3·96 (W.)	3·69 (V.S.)	3·43 (V.S.)
3·19 (V.W.)	3·04 (M.)	2·94 (W.)	2·79 (M.)	2·65 (V.W.)	2·61 (V.W.)
2·47 (V.W.)	2·42 (W.)	2·36 (W.)	2·28 (W.)	2·16 (V.W.)	2·11 (V.W.)

(*Needle form*).

5·89 (F.S.)	5·01 (S.)	4·82 (S.)	4·18 (F.S.)	3·72 (V.W.)	3·45 (V.S.)
3·28 (F.S.)	3·12 (F.S.)	2·90 (M.)	2·41 (W.)	2·34 (M.)	

Acetate of compound B.

13·80 (S.)	8·34 (S.)	7·00 (W.)	6·49 (W.)	5·87 (W.)	5·08 (W.)
4·73 (F.S.)	4·27 (S.)	3·94 (W.)	3·78 (W.)	3·61 (W.)	3·49 (F.S.)
3·24 (M.)	3·08 (V.W.)	2·92 (W.)	2·79 (V.W.)	2·70 (V.W.)	2·59 (V.W.)

*Gallocatechin.**

6·25 (F.S.)	5·59 (F.S.)	5·24 (V.W.)	4·85 (S.)	4·45 (F.S.)	4·11 (M.)
3·82 (S.)	3·68 (W.)	3·43 (V.S.)	3·23 (V.W.)	3·11 (F.S.)	2·86 (F.S.)
2·60 (M.)	2·36 (W.)	2·28 (W.)	2·18 (V.W.)	2·09 (M.)	

Gallocatechin hexa-acetate.

11·06 (F.S.)	7·04 (S.)	6·60 (S.)	5·72 (F.S.)	5·32 (V.W.)
4·95 (M.)	4·78 (M.)	4·36 (V.S.)	4·07 (M.)	3·88 (M.)
3·69 (M.)	3·40 (F.S.)	3·21 (V.W.)	3·08 (V.W.)	2·87 (M.)
2·74 (V.W.)	2·64 (V.W.)	2·51 (V.W.)	2·43 (V.W.)	2·33 (V.W.)

* A different Debye–Scherrer pattern was obtained from one particular specimen of gallocatechin, obtained early in the investigation, presumably a dimorphic form. The pattern given is that observed with all later specimens.

X-Ray Determination of Molecular Weights.—(a) *Substance B.* *X*-Ray measurements were made on a Unicam goniometer with a 6-cm. diameter cylindrical camera, employing copper-$K\alpha$, nickel-filtered radiation (wave length = 1·5387kX) from a tube operated at 30 kv. and 15—20 ma. The fibrous crystals of substance *B* (cross section $< 1\ \mu$) were too small for the production of single-crystal oscillation photographs, and as the fibres did not exhibit parallel growth, fibre patterns could not be obtained. The prismatic crystals of substance *B* were extremely small (0·03 × 0·03 × 0·15 mm.), and the cell dimensions and space-group were therefore determined by analysis of a set of oscillation photographs, obtained by oscillating the crystal through 10° angles at 10° intervals about the axis of elongation (the *c* axis). The mean exposure time was in the region of 14 hours. As the high-angle reflections near the fiducial marks were not resolved, an error of 0·5% is allowed in each of the values of $a \sin \beta$, b, and c, and of 1·5% in the calculated molecular weight. The crystals were examined for the presence of a centre of symmetry by the pyroelectric test. The monoclinic angle was determined by interpretation of reflected spots on the sixth layer lines. The density of the crystals was determined by the flotation method, diluted portions of Thoulet's solution being used. It was best to observe the movements in the liquids of the very small single crystals in polarised light between crossed Nicols. It was found that the prismatic crystals of substance *B* had a monoclinic unit cell with $a \sin \beta = 10{\cdot}73 \pm 0{\cdot}05\ kX$, $b = 10{\cdot}20 \pm 0{\cdot}05\ kX$, $c = 12{\cdot}66 \pm 0{\cdot}06\ kX$, $\beta = 97°\ 30'$. Density = 1·545 ± 0·002 g./c.c. Space-group $P2_1/n$. Number of molecules per unit cell = 4. Hence the molecular weight of substance *B* is calculated to be 324 ± 5.

(b) *Acetyl derivative of substance B.* The fibrous needles of this substance were too small for single-crystal oscillation photographs to be obtained. Crystallised from water–alcohol, the fibres formed bundles with their long axes parallel. From the fibre pattern obtained by oscillating such a bundle about the common axis, the dimensions of the unit cell were found to be approximately 14·8 × 17·1 × 5·5 kX. The density of the crystals was 1·37 ± 0·01 g./c.c. The fibres appeared to be weakly pyroelectric. As, also, the crystals show slightly inclined extinction, a monoclinic space-group without a centre of symmetry has been assumed, with two molecules per unit cell. On this assumption, the molecular weight is calculated to be 577 : an accuracy of only about 16% can be claimed for this value.

(c) *Gallocatechin hexa-acetate.* The molecular weight of this substance was determined exactly as for substance *B*, except that, with somewhat larger crystals (0·5 × 0·5 × 0·012 mm.) a shorter exposure time (approx. 2 hours) was required. The lamellar crystals had a monoclinic unit cell with $a \sin \beta = 13{\cdot}12 \pm 0{\cdot}07\ kX$, $b = 9{\cdot}00 \pm 0{\cdot}05\ kX$, $c = 11{\cdot}53 \pm 0{\cdot}06\ kX$, $\beta = 107°$. Density = 1·355 ± 0·002 g./c.c. Space-group $P2_1$. Number of molecules per unit cell = 2. Hence the molecular weight of this hexa-acetate is calculated to be 559 ± 9. The fibrous needles of gallocatechin itself were too small for the production of single-crystal oscillation photographs, and as the fibres did not exhibit parallel growth, fibre patterns could not be obtained.

The work was carried out partly under the auspices of the Research Committee of the Indian Tea Association and partly as an investigation by the Laboratories of J. Lyons & Co. Ltd.

The authors thank Dr. Lampitt for his continued interest in this work.

INDIAN TEA ASSOCIATION.
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[*Received, April 25th,* 1946.]