148. *The Structures of Buddleoflavonoloside (Linarin) and of Buddleoflavonol (Acacetin).*

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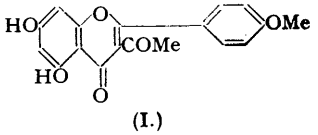
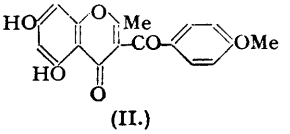
Buddleoflavonoloside, a glycoside isolated from *Buddleia variabilis*, gives L-rhamnose, D-glucose, and acacetin (III) on acid hydrolysis. This aglycone had been previously incorrectly regarded as 3-acetylacacetin. Hydrolysis of the methylated glycoside gave 5-methylacacetin, 2 : 3 : 4-trimethyl L-rhamnose, and 2 : 3 : 4-trimethyl D-glucose, thus establishing the structure of buddleoflavonoloside as 7-rutinosylacacetin (VII), a glycoside which had previously been found to occur in toad flax, *Linaria vulgaris*, and had been termed linarin.

Yü (*Bull. Soc. Chim. biol.*, 1933, **15**, 483) isolated from the leaves, buds, and flowers of the shrub, *Buddleia variabilis* Hemsl. var. grandiflora, a glycoside, buddleoflavonoloside, for which the formula $C_{30}H_{34}O_{15}$ was proposed. Hydrolysis of this glycoside was reported to give an aglycone, buddleoflavonol, $C_{18}H_{14}O_6$, L-rhamnose, and D-glucose. The position of attachment and the structure of the disaccharide residue were not determined.

According to Yü, buddleoflavonol is 3-acetyl-5 : 7-dihydroxy-4'-methoxyflavone (3-acetylacacetin) (I), but it occurred to us that if his observations were correct the alternative, isomeric structure, 3-anisoyl-5 : 7-dihydroxy-2-methylchromone (II) was equally possible on the evidence available (cf. Baker, *J.*, 1933, 1381). Recently, Baker and Butt (*J.*, 1949, 2142; see also Baker and Glockling, *J.*, 1950, 2759) have shown that it is possible to differentiate between such isomeric compounds by reaction with benzylamine, and it was, therefore, considered opportune to reinvestigate the structures of buddleoflavonol and of buddleoflavonoloside.



(I.)



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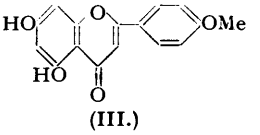


(III.)

We have found that a glycoside, obviously identical with that described by Yü, can be easily extracted from the leaves, flowers, or buds of *Buddleia variabilis* and gives a well-crystalline aglycone on acid hydrolysis. This phenolic aglycone was found to be represented by the formula $C_{15}H_7O_2(OH)_2(OMe)$; it gave a reddish-brown ferric chloride reaction, was readily converted into diacetyl and dimethyl derivatives, and on demethylation yielded a trihydroxy-compound, characterised as its triacetyl derivative. Comparison of the properties of the aglycone and its derivatives with those of acacetin (5 : 7-dihydroxy-4'-methoxyflavone) (III) and its derivatives (see Table I) showed undoubted identity.

Table I.

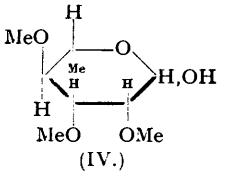
	Buddleoflavonol. M. p.	Acacetin. M. p.	
	263° [a]; 265° [b]	263° [a]; 261° [c]	[a] This paper. [b] Yü (*loc. cit.*).
Diacetyl derivative	204° [a]	204° [a]; 203° [c]	Robinson and Venkataraman (*J.*,
Dimethyl derivative	156° [a]	156° [d]	1926, 2348). [d] Czajkowski, Kos-
Demethyl derivative	346—348° [a]	347° [d]	tanecki, and Tambor (*Ber.*, 1900,
Triacetyl demethyl derivative	182° [a]	181—182° [d]	**33**, 1991).

Yü states that the aglycone gives a positive iodoform test, but we have been unable to confirm this. He also claims that alkaline hydrolysis of the aglycone gave acetone, which was detected by colour tests. We were unable to detect the liberation of acetone during the alkaline hydrolysis of the aglycone when the volatile products were passed into an acid solution of 2 : 4-dinitrophenylhydrazine.

The sugars formed by hydrolysis of buddleoflavonoloside were identified as L-rhamnose and D-glucose, which were separated by partition chromatography on a column of hydrocellulose, using *n*-butanol–ethanol–water as the mobile phase. With the aid of an automatic receiver changer (Hough, Jones, and Wadman, *J.*, 1949, 2511) the eluate was separated into two portions, one containing L-rhamnose, characterised as its *N*-benzoylhydrazone, and the other containing D-glucose, characterised as its β-penta-acetate. Buddleoflavonoloside gave

a strong ferric chloride reaction, showing that the 5-hydroxyl group was free, so that the sugars must be present as a disaccharide residue attached to the oxygen atom in position 7. This was confirmed by an examination of the completely methylated buddleoflavonoloside.

Buddleoflavonoloside was treated with methyl sulphate and aqueous sodium hydroxide, giving a hepta-*O*-methyl derivative which, on acid hydrolysis, gave 7-hydroxy-5 : 4′-dimethoxyflavone (acacetin 5-methyl ether) (VI) and a mixture of methylated sugars. Acacetin 5-methyl ether has not previously been obtained in a state of purity; it was characterised as the acetate. The mixture of methylated sugars was separated by partition chromatography (Hough, Jones, and Wadman, *loc. cit.*), giving mainly 2 : 3 : 4-trimethyl L-rhamnose (IV), and 2 : 3 : 4-trimethyl D-glucose (V), which were both characterised as their crystalline anilides.



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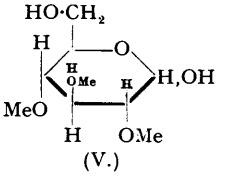

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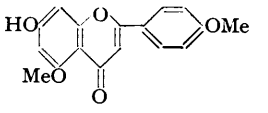
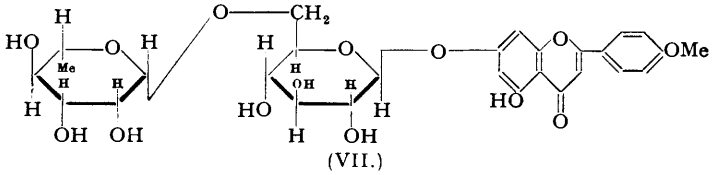


(VI.)



(VII.)

It follows that the disaccharide residue is derived from 6-(L-rhamnopyranosyl)-D-glucopyranose. The disaccharide rutinose is 6-(β-L-rhamnopyranosyl)-D-glucose, so it was considered probable that buddleoflavonoloside was 7-rutinosylacacetin. Previously the glycoside, linarin, isolated from the flowers of toad flax, *Linaria vulgaris* L., had been identified as 7-rutinosylacacetin (Merz and Wu, *Arch. Pharm.*, 1936, **274**, 126; Zemplén and Bognár, *Ber.*, 1941, **74**, 1818), and comparison of the physical properties of natural and synthetic linarin with those of buddleoflavonoloside given in Table II shows that buddleoflavonoloside and linarin are undoubtedly identical, though direct comparison of specimens has not been possible. The only apparent

TABLE II.

Compound	M. p.	$[\alpha]_D$ in acetic acid.	$[\alpha]_D$ in pyridine.
Buddleoflavonoloside	262—263° [a]; 274—276° [b] *	−98·6° [a]; −97·35° [b]	−89·6° [a]; −101·7° [b]
Natural linarin	265° [c]	−100·1° [c]	
Synthetic linarin	263—264° [d]	−100° [d]	−87·3° [d]; −88·5° [d]
Hepta-acetyl derivative of		in benzene.	
Buddleoflavonoloside ...	126—134° [a]	−70·3° [a]	—
Natural linarin	123—125° [c]	−70·8° [c]	—
Synthetic linarin	120—126° [d]	−71·1° [d]	—

[a] This paper. [b] Yü (1933). [c] Merz and Wu (1936). [d] Zemplén and Bognár (1941).
* Maquenne block.

difference between the buddleoflavonoloside isolated in this work, that isolated by Yü, and linarin, lies in their degree of hydration. Our analytical figures agree more closely with those required for a sesquihydrate than for a monohydrate, but the compound does not lose water at 184°; Yü does not record the presence of water of crystallisation; Merz and Wu state that linarin is a monohydrate which does not lose its water of crystallisation at 100°, whereas Zemplén and Bognár describe synthetic linarin as a monohydrate which is dehydrated at 138°.

The synthesis of linarin by Zemplén and Bognár (*loc. cit.*) from hepta-acetyl α-rutinosyl bromide (α-acetobromorutinose) and acacetin, followed by hydrolysis of the intermediate hepta-acetyl derivative, showed the β-configuration of the glycosidic linkage between the rutinose and the acacetin residues. Acetylation of buddleoflavonoloside gave a hepta-acetyl derivative which was identical with hepta-acetyl linarin (see Table II). Both buddleoflavonoloside and linarin are, therefore, represented by the formula (VII).

In view of these findings we propose that the names buddleoflavonoloside and buddleoflavonol should be no longer used, and should be replaced by linarin and acacetin respectively.

Experimental.

M. p.s are uncorrected. Analyses are by Mr. W. M. Eno, Bristol, and Drs. Weiler and Strauss, Oxford.

Isolation of Buddleoflavonoloside.—(*a*) Various parts of the shrub, *Buddleia variabilis*, were extracted and a typical experiment is given. The method first used is based upon that described by Yü (*loc. cit.*). Freshly collected leaves (300 g.) were well chopped and heated under reflux with ethanol (2·4 l.) and water (600 c.c.) for 2·5 hours. After filtration, most of the ethanol was removed by distillation under diminished pressure, and chlorophyll was removed from the aqueous residue by filtration and extraction with ether (2 × 1 l.). After being kept in a refrigerator overnight, the micro-crystalline precipitate (1·5 g., 0·5%), m. p. 260—262° (decomp.), was collected. Five crystallisations from ethanol (*ca.* 350 c.c.)–water (*ca.* 350 c.c.) gave buddleoflavonoloside (360 mg.) as cream-coloured microscopic needles, m. p. 262—263° (dependent upon rate of heating), $[\alpha]_D^{19}$ −98·6° (*c*, 0·28 in acetic acid), $[\alpha]_D^{15}$ −89·6° (*c*, 0·31 in pyridine) (Found, in material dried at 80°: C, 54·4; H, 5·7; OMe, 5·8. In material dried at 138°: C, 54·5; H, 5·7; OMe, 6·2. In material dried at 184°: C, 54·2; H, 6·0. Calc. for $C_{28}H_{32}O_{14},H_2O$: C, 55·0; H, 5·6; OMe, 5·1%. Calc. for $C_{28}H_{32}O_{14},1\frac{1}{2}H_2O$: C, 54·3; H, 5·65; OMe, 5·0%). An ethanol solution of buddleoflavonoloside gives a strong greenish-brown ferric chloride reaction. Similar extractions of freshly collected buds (300 g.) gave the same glycoside (average yield, 5·8 g., 1·9%) and from the freshly collected flowers (300 g.) the average yield was 5·9 g. No advantage was gained by drying these plant materials before extraction.

(*b*) The following milder method gave a purer final product. Freshly picked flowers (2 kg.) and acetone (11·5 l.) were left at room temperature for 24 hours, then filtered, and the filtrate was concentrated under diminished pressure. The aqueous residue was left in a refrigerator overnight and the brown solid collected by centrifuging. After very thorough washing with ethanol and then ether, the glycoside was obtained as cream-coloured, microcrystalline needles (9·53 g., 0·5%), m. p. 266—267° (decomp.).

Hepta-acetyl Buddleoflavonoloside.—Buddleoflavonoloside (0·3 g.), anhydrous pyridine (4 c.c.) and acetic anhydride (4 c.c.) were heated at 85—95° for 3 hours, cooled, and poured into water. The precipitate was collected, washed, dried (yield, 0·43 g.), and recrystallised from 50% aqueous ethanol, giving *hepta-acetyl buddleoflavonoloside* (0·25 g.) as colourless, crystalline aggregates, m. p. 126—134° (unaltered by further recrystallisation), $[\alpha]_D^{15}$ −58·7° (*c*, 0·56 in pyridine), $[\alpha]_D^{18}$ −70·3° (*c*, 0·27 in benzene) [Found, in material dried over phosphoric anhydride at 80° under diminished pressure: C, 56·6; H, 5·6; OMe, 3·7; Ac, 34·5. $C_{27}H_{22}O_6(OMe)(OAc)_7$ requires C, 56·9; H, 5·2; OMe, 3·5; Ac, 34·0%].

Deacetylation of Hepta-acetyl Buddleoflavonoloside.—After a solution of hepta-acetyl buddleoflavonoloside (100 mg.) had been kept in 0·1N-methanolic sodium methoxide (25 c.c.) at 0° for 24 hours, it was neutralised with dilute hydrochloric acid and the precipitated solid collected and dried (53 mg., 78%), m. p. 244—248° (decomp.). Recrystallisation from ethanol–water as before gave buddleoflavonoloside, m. p. and mixed m. p. 270° (decomp.) (Found: C, 54·2; H, 5·9%).

Hydrolysis of Buddleoflavonoloside. Isolation of 5 : 7-Dihydroxy-4′-methoxyflavone (Acacetin) and Identification of L-Rhamnose and D-Glucose.—Buddleoflavonoloside (3 g.) and 3% sulphuric acid (1 l.) were heated under reflux for 18 hours and left overnight. The crystalline precipitate (1·45 g., 100%), m. p. 263°, was collected and recrystallised from ethanol, giving the aglycone, 5 : 7-dihydroxy-4′-methoxyflavone (acacetin), as yellow needles, m. p. 263° (Found: C, 67·3; H, 4·3; OMe, 10·8. Calc. for $C_{15}H_9O_4{\cdot}OMe$: C, 67·6; H, 4·2; OMe, 10·9%). It showed no depression of m. p. when mixed with synthetic acacetin prepared as described by Robinson and Venkataraman (*J.*, 1926, 2348), who give m. p. 261°. It gave a reddish-brown ferric chloride reaction in ethanol. The diacetyl derivative, prepared by boiling the aglycone (1·0 g.) under reflux for 1 hour with anhydrous sodium acetate (3 g.) and acetic anhydride (20 c.c.) and then pouring the mixture into water (yield 1·15 g.; m. p. 203°), had m. p. and mixed m. p. with an authentic specimen of diacetyl acacetin, 204°, after crystallisation (needles) from benzene (50 c.c.) (Found: C, 65·1; H, 4·8; OMe, 8·4. Calc. for $C_{19}H_{13}O_6{\cdot}OMe$: C, 65·2; H, 4·4; OMe, 8·4%) (Robinson and Venkataraman, *loc. cit.*, give m. p. 203°).

Excess of barium carbonate was added to the acid filtrate and, after being shaken, the neutral solution was filtered and then concentrated under diminished pressure, and traces of inorganic material were removed by addition of charcoal and filtration. Complete evaporation under diminished pressure gave a syrupy mixture *M* of sugars (1·45 g.; 87% calculated on the formula $C_{16}H_{11}O_5{\cdot}C_{12}H_{21}O_9,1{\cdot}5H_2O$). A very small portion of this material was separated on a paper chromatogram (Partridge and Westall, *Biochem. J.*, 1948, **42**, 238; Flood, Hirst, and Jones, *J.*, 1948, 1679), and the sugars were located by spraying with aniline phthalate (Partridge, *Nature*, 1949, **164**, 443; Hough, Jones, and Wadman, *J.*, 1950, 1702). A mixture of D-glucose and L-rhamnose, run on the same paper, gave a precisely similar chromatogram.

Isolation of L-Rhamnose and D-Glucose from Hydrolysate of Buddleoflavonoloside.—The mixture of sugars *M* (above) was separated (see Hough, Jones, and Wadman, *J.*, 1949, 2511) on a column of powdered hydrocellulose (65 × 3·5 cm.). After washing of the column with ethanol–water (1 : 1), the sugar mixture was dissolved in the minimum quantity of water, placed on the top of the column, and when it had soaked into the hydrocellulose the column was developed with a mixture of *n*-butanol saturated with water (9 parts) and ethanol (1 part). The eluate was collected in about 5-c.c. portions, by means of an automatic receiver changer (Hough, Jones, and Wadman, *loc. cit.*). The contents of each tenth receiver were evaporated and the residue examined on a paper chromatogram using Whatman No. 1 paper and, as the mobile phase the upper layer of the mixture, *n*-butanol (50 parts), water (40 parts), and ethanol (10 parts). Tubes 90—150 contained sugar *A* (R_G 0·30), and tubes 240—360 sugar *B* (R_G 0·09).

The combined contents of tubes 90—150 were evaporated under diminished pressure, and the syrup

(sugar *A*) (583 mg., 70%, calc. on one molecule of rhamnose per glycoside molecule) was crystallised by warming it with acetone (10 c.c.)–water (0·75 c.c.), and after several days the large, colourless rhombs of L-rhamnose monohydrate (441 mg.) were collected. These had m. p. and mixed m. p. with an authentic specimen, 100°, and $[\alpha]_D^{19}$ +8·1° (*c*, 1·08 in water) (Walton, *J. Amer. Chem. Soc.*, 1921, **43**, 127, gives for L-rhamnose hydrate, $[\alpha]_D^{20}$ +8·5°), and gave an *X*-ray powder photograph identical with that given by L-rhamnose hydrate (*X*-ray photographs kindly taken by Dr. T. H. Bevan). It was further characterised as L-rhamnose benzoylhydrazone, m. p. and mixed m. p. with an authentic specimen, 188° (decomp.; m. p. dependent upon rate of heating) (cf. Hirst, Jones, and Woods, *J.*, 1947, 1048, who record m. p. 180°, and Hirst, Hough, and Jones, *J.*, 1949, 3148, who give m. p. 183°).

The combined contents of tubes 240—360 yielded a syrup (sugar *B*) (637 mg., 70% calc. on one molecule of glucose per glycoside molecule) which was crystallised from water (1·5 c.c.) and acetone (10 c.c.). This D-glucose, after being dried over phosphoric anhydride under diminished pressure at 56°, had m. p. and mixed m. p. 146°, $[\alpha]_D^{19}$ (after 24 hours) +50·8° (*c*, 0·63 in water) $[\alpha]_D$ (equilibrium value) for D-glucose +52·8°. This D-glucose was further characterised as the β-penta-acetate, m. p. and mixed m. p. 130°.

5 : 7 : 4′-*Trimethoxyflavone*.—Methyl sulphate (3 c.c.) in acetone (20 c.c.) and 30% aqueous sodium hydroxide (13 c.c.) were added simultaneously to a vigorously stirred solution of the aglycone (200 mg.) in 30% aqueous sodium hydroxide (7 c.c.) at 40—50°. After 0·5 hour water was added and the light brown solid (179 mg., 81%) was collected and crystallised from ethanol (5 c.c.), giving 5 : 7 : 4′-trimethoxyflavone as colourless needles, m. p. 156°. Czajkowski, Kostanecki, and Tambor (*Ber.*, 1900, **33**, 1991) give m. p. 156° for trimethylapigenin.

5 : 7 : 4′-*Trihydroxyflavone* (*Apigenin*).—The aglycone (300 mg.) was heated under reflux with glacial acetic acid (15 c.c.) and hydrobromic acid (15 c.c.; *d* 1·5) for 8 hours, then poured into water, and the crystals (266 mg., 93%), m. p. 346—348°, were collected. This 5 : 7 : 4′-trihydroxyflavone (150 mg.) was characterised by heating it under reflux with acetic anhydride (4·5 c.c.) and fused sodium acetate (700 mg.), giving 5 : 7 : 4′-triacetoxyflavone (203 mg., 97%) as cream-coloured needles, m. p. 182°, from ethanol (16 c.c.)–water (6 c.c.). Czajkowski, Kostanecki, and Tambor (*loc. cit.*) give m. p. 347° for apigenin, and m. p. 181—182° for triacetylapigenin.

Methylation of Buddleoflavonoloside and Hydrolysis of the Product. Isolation of 7-*Hydroxy*-5 : 4′-*dimethoxyflavone* (VI).—Methyl sulphate (60 c.c.) in acetone (180 c.c.), and 30% aqueous sodium hydroxide (180 c.c.) were added dropwise and simultaneously in five portions at two-hourly intervals to a stirred solution of buddleoflavonoloside (6 g.) in 30% sodium hydroxide (120 c.c.) at room temperature. Two hours after the last additions, the mixture was accurately neutralised with dilute hydrochloric acid, and the acetone removed under diminished pressure. The residue was extracted with chloroform (3 × 300 c.c.), and the extracts were washed with water, dried ($MgSO_4$), and evaporated, leaving crude hepta-*O*-methylbuddleoflavonoloside as a light brown product (5·7 g.), m. p. *ca.* 114°, which could not be crystallised [Found: C, 61·4; H, 7·1; OMe, 32·2. Calc. for $C_{27}H_{22}O_6(OMe)_8$: C, 60·9; H, 6·8; OMe, 35·9%]. This was then dissolved in glacial acetic acid (125 c.c.), 5% hydrochloric acid (250 c.c.) added, and the mixture heated on a steam-bath for 5 hours. After being cooled, the yellow, crystalline precipitate was collected, washed, and dried (2·12 g., 86%), m. p. 260—264° (decomp.); it gave no ferric chloride reaction. Crystallisation from ethanol (500 c,c.) (charcoal) gave 7-hydroxy-5 : 4′-dimethoxyflavone as yellow, rectangular prisms (970 mg.), m. p. 298° (decomp.) [Found: C, 68·4; H, 4·6; OMe, 20·1. $C_{15}H_8O_3(OMe)_2$ requires C, 68·4; H, 4·7; OMe, 20·8%]. Concentration of the ethanolic mother-liquors gave a second fraction (500 mg.), m. p. 294° (decomp.). This substance has not previously been obtained pure. Zemplén and Bognár (*Ber.*, 1941, **74**, 1820) give m. p. 268—268·5° for acacetin 5-methyl ether, and Narasimhachari and Seshadri (*Proc. Indian Acad. Sci.*, 1949, **30** [A], 158) give m. p. 265—267°. Vongerichten (*Ber.*, 1900, **33**, 2909) records m. p. 264°, but it seems probable that his material was acacetin (see below).

This 7-hydroxy-5 : 4′-dimethoxyflavone (200 mg.) was heated at 100° for 1 hour with acetic anhydride (8 c.c.) and fused sodium acetate (300 mg.). The solid (235 mg., 98%), m. p. 152°, obtained on pouring the mixture into ice–water (80 g.), was collected, and crystallised from aqueous ethanol and then from aqueous acetic acid, giving 7-*acetoxy*-5 : 4′-*dimethoxyflavone* as colourless needles, m. p. 152° [Found: C, 66·6; H, 5·1; OMe, 17·7. $C_{16}H_{10}O_4(OMe)_2$ requires C, 67·1; H, 4·7; OMe, 18·2%]. According to Vongerichten (*loc. cit.*) this compound has m. p. 204°, but his material was probably diacetylacacetin, which we find has m. p. 204°.

Isolation of 2 : 3 : 4-*Trimethyl* L-*Rhamnose* (IV) *and* 2 : 3 : 4-*Trimethyl* D-*Glucose* (V).—After separation of the 7-hydroxy-5 : 4′-dimethoxyflavone from the acid hydrolysate (above), the filtrate was treated with excess of saturated, aqueous lead acetate, and filtered from lead chloride, hydrogen sulphide passed in, and the filtrate concentrated to a small volume under diminished pressure. Water was then added and removed several times under diminished pressure, to remove most of the acetic acid, and the solution was finally treated with Amberlite resin IR4B until the pH was 7, after which the solution was concentrated under diminished pressure, giving a syrup. This product was separated on a column of hydrocellulose as previously described for the sugar mixture *M* (above). The solvent mixture used for development was *n*-butanol (30 parts) and light petroleum (b. p. 80—100°; 70 parts), and the contents of every tenth tube were concentrated and examined on a paper chromatogram, using as the developing solvent the upper layer of a mixture of *n*-butanol (50 parts), water (40 parts), and ethanol (10 parts). The sugar spots were located by spraying the dried papers with 1% solution of aniline phthalate in *n*-butanol and gently warming.

Tubes 41—80 contained material which gave a greyish spot (R_G 1·01), tubes 91—130 contained material which gave a red spot (R_G 1·00), tubes 131—210 contained at least three substances which gave a red spot (R_G 1·00), a grey spot (R_G 0·86), and a reddish-brown spot (R_G 0·85), tubes 211—370 contained material which gave a reddish-brown spot (R_G 0·85).

The contents of tubes 41—80 were combined and the solvent was removed under diminished pressure, giving a syrup (723 mg., 55% calc. on one trimethyl rhamnose residue per heptamethyl buddleoflavonoloside molecule). This syrup could not be crystallised, but its specific rotation, $[\alpha]_D^{16}$ +22° (*c*, 3·5 in water), and its rate of movement on the paper chromatogram indicated that it was 2 : 3 : 4-trimethyl rhamnose. Hirst and Macbeth (*J.*, 1926, 25) give $[\alpha]_D$ +24·9° for 2 : 3 : 4-trimethyl rhamnose. It was characterised as its anilide (F. Smith, *J.*, 1940, 1046) which, after several recrystallisations from light petroleum (b. p. 80—100°), was obtained as needles, m. p. 119°. A mixed m. p. with an authentic specimen, m. p. 122°, kindly supplied by Dr. J. K. N. Jones, was 120°. Smith (*loc. cit.*) gives m. p. 111° for 2 : 3 : 4-trimethyl L-rhamnose anilide.

Similarly, tubes 91—130 gave a residue (73 mg.) which, from its rate of movement and direct comparison on a paper chromatogram, was 2 : 3 : 4 : 6-tetramethyl glucose. It was not further investigated. Tubes 131—210 gave a very small amount of material which, from its behaviour on a paper chromatogram, was not homogeneous. This fraction contained at least three sugars, 2 : 3 : 4 : 6-tetramethyl glucose (R_G 1·00), a dimethyl rhamnose (R_G 0·86), and 2 : 3 : 4-trimethyl glucose (R_G 0·85). This fraction was not further investigated.

The contents of tubes 211—370 were combined and gave a non-crystalline syrup (1·014 g., 71% calc. on one trimethyl glucose residue per heptamethylbuddleoflavonoloside molecule), $[\alpha]_D^{18}$ +67·3° (equilibrium value) (*c*, 5·3 in water). For 2 : 3 : 4-trimethyl glucose, Hess and Gramberg (*Ber.*, 1939, **72**, 1905) give $[\alpha]_D^{20}$ +62·4° in water, and Irvine and Oldham (*J.*, 1921, **119**, 1756) give $[\alpha]_D^{20}$ +66·8° (equilibrium value; *c*, 4·086 in water). This syrup was compared on the same paper chromatogram with authentic specimens of 2 : 3 : 4- and 2 : 3 : 6-trimethyl D-glucose. Its rate of movement was identical with that of 2 : 3 : 4-trimethyl D-glucose. This identity was confirmed by its characterisation as 2 : 3 : 4-trimethyl D-glucose anilide, white needles, m. p. 144°, from light petroleum (b. p. 80—100°). Peat, Schlüchterer, and Stacey (*J.*, 1939, 584) give m. p. 145—146° for 2 : 3 : 4-trimethyl D-glucose anilide.

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