594. *Anthelmintics: Kousso. Part II.* The Structures of Protokosin, α-Kosin, and β-Kosin.*

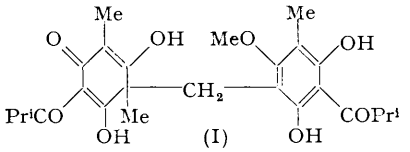
By A. J. Birch and A. R. Todd.

New formulæ are proposed for protokosin (I, or a tautomer) and α- (II) and β-kosin (III) which are based chiefly on absorption spectra and reduction of the kosins with sodium amalgam to methylenebismethylphloroglucinol derivatives and *iso*butaldehyde.

The isolation of protokosin from kousso (*Hagenia abyssinica*) flowers was described in Part I,* together with some degradative studies on the compound. The available evidence was sufficient to indicate that protokosin was related in structure to some of the methylenebisphloroglucinol derivatives, *e.g.*, aspidin (V), isolated by Boehm from *Aspidium filix mas*

* Part I, Hems and Todd, *J.*, 1937, 562.

(cf., *e.g.*, *Annalen*, 1903, **329**, 321), and for it a formula $C_{22}H_{28}O_7$ (IV) was suggested as a basis for further work. Further investigation of protokosin has been severely hampered by lack of material. The original source was a commerical sample of kousso of unknown origin. More recently, through the kind co-operation of the Colonial Products Research Council who arranged for plant collection, authentic samples of dried flowers, male and female, of *Hagenia abyssinica* have been obtained from various localities in East Africa; from only one of these was it possible to isolate crystalline protokosin and even there the yield was very low, although extracts of the flowers contained large amounts of amorphous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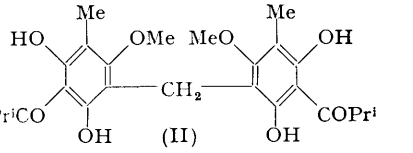
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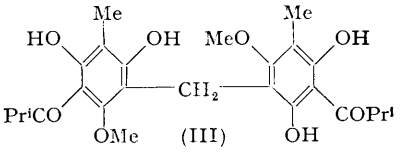
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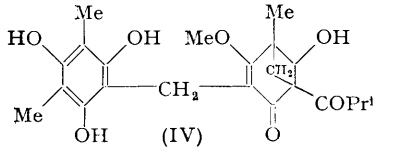
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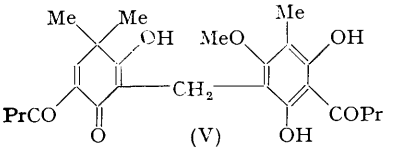
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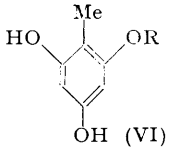
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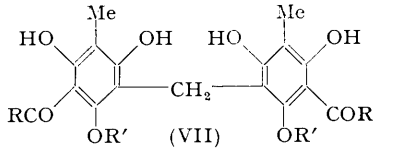
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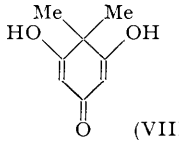
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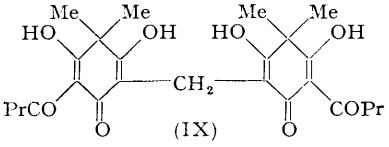
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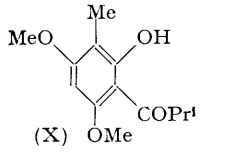

(X)

material some of which could be converted into kosins by the action of alkali (Rivett, Thesis, Cambridge 1948). Studies of these complex materials are as yet very incomplete, but we have recently re-examined the known facts about protokosin in the light of some further experimental results and have reached certain conclusions which are now presented.

The published evidence relating to the structure of protokosin can be summarised as follows. On reduction with zinc dust and sodium hydroxide it gave rise to *iso*butyric acid and a mixture of methylphloroglucinols from which was isolated 1 : 3 : 5-trimethylphloroglucinol, together with the compounds α- and β-kosin which are isomeric with the starting material but possess two methoxyl groups instead of one. Protokosin gave rise on alkali-fusion to methylphloroglucinol (VI; R = H), and it had been observed by Lobeck (*Arch. Pharm.*, 1901, **239**, 672) that α-kosin on vigorous treatment with alkali or acid produced some methylphloroglucinol β-methyl ether (VI; R = Me). Protokosin is optically active and the kosins are inactive. It was considered that the latter are derived from the former by migration of a methyl group from carbon to oxygen. We now consider that the molecular formula of protokosin should be revised to $C_{25}H_{32}O_8$ and that the most adequate structures are (I) for protokosin, (II) for α-kosin, and (III) for β-kosin.

The suggestion that a considerable alteration might have to be made to (IV) was first put forward by Mulholland (Thesis, Manchester, 1947) who pointed out that the ultra-violet absorption spectra of protokosin and the kosins could not be reconciled on the basis of (IV) with those of a number of model compounds. The absorptions of some relevant compounds are given in the Table.

It is apparent that compounds containing an acylphloroglucinol nucleus possess between 200 and 300 mμ a maximum at 223—233 mμ and another at 271—293 mμ, the former having a greater regularity of position and intensity. The intensity at the shorter wave-length in

	$\lambda_{max.}$ (mμ)	$\varepsilon_{max.}$	$\lambda_{min.}$ (mμ)	Ref.
A, Methylphloroglucinol	270·5	623	254	1
B, Dimethylphloroglucinol	277	1,070	254	1
C, Trimethylphloroglucinol	271	900	—	2
	274	885		
D, Phloro*iso*butyrophenone	229·3	12,800	247·5	1
	289	16,000		
E, *C*-Acetyldimedon	231	10,000	250	3
	277	10,000		
F, (VII; R = Me, R′ = H)	227	21,800	—	1
	291	25,760		
G, (VII; R = Pr^i, R′ = Me)	233	27,700	—	4
	292·5	24,700		
H, Filicinic acid (VIII)	244	15,100	—	4
	283	4,350		
J, Albaspidin (IX)	223	27,300	—	4
	271	13,400		
K, Baeckeol (X)	227	10,300	250	1
	293	17,300		
L, Flavaspidic acid	229	26,350	255	1
	292·5	20,300		
M, Protokosin	223	25,860 †	253	
	287	19,840 †		
N, α-Kosin	230	30,000 †	254	1
	287	23,000 †		
O, β-Kosin	228	30,300 †	254·5	1
	292	21,260 †		
P, Methylenebisbaeckeol	Inflex. 232	23,880	250	
	Max. 286	28,000		

1, Mulholland, *loc. cit.*; 2, Campbell and Coppinger, *J. Amer. Chem. Soc.*, 1951, **73**, 2708; 3, Birch, *J.*, 1951, 3026; 4, Dr. T. H. Quibell, personal communication.
† Calculated on C_{25} formula.

compounds with one nucleus is 10,000—13,000 (D, E, K) and in those with two nuclei insulated by a methylene group (F, G, J, and probably L) is 22,000—27,000. An unacylated nucleus absorbs with a much lower intensity (A, B, C), and, although the non-aromatic compound (H) has a high-intensity band in the region concerned, this is at a higher wave-length (244 mμ) than the *ca.* 230-mμ band. Evidently, if protokosin and the kosins (M, N, O) have two acylphloroglucinol nuclei their absorption spectra possess maxima of about the right positions and intensities. It is difficult to reconcile their spectra with a formula (IV) containing one unacylated nucleus. The two bands seem to be characteristic of the enolised β-triketone system and they are found even with the non-aromatic compounds (E, J); the triketone system in (IV) cannot enolise because of the bridge.

Another observation which is not in accord with (IV) is the production of two mols. of *iso*butyric acid on hydrolysis with sulphuric acid (Leichsenring, *Arch. Pharm.*, 1890, **232**, 50; Rivett, *loc. cit.*) although the fact that the acid was estimated merely by titration might permit of other explanations. The only likely formula which fits the analytical figures and allows the presence of two *iso*butyrylmethylphloroglucinol nuclei is $C_{25}H_{32}O_8$ [Found, for protokosin: C, 65·4, 65·2, 65·4; H, 7·0, 7·0, 7·0; MeO, 8·0, 8·1; 8·1%; *M* (Rast), 412, 396, 399. Found, for α-kosin: C, 65·1, 65·3; H, 7·0, 6·8; MeO, 13·2, 13·5. Found, for β-kosin: C, 65·3; H, 7·0; MeO, 13·4%; *M*, 460 (Hems and Todd, *loc. cit.*). Calc. for $C_{25}H_{32}O_8$: C, 65·2; H, 7·0; MeO, 6·8; 2MeO, 13·4%; *M*, 460]. The molecular-weight determinations are about 15% low for the C_{25}-formula, but this error is not exceptional for Rast determinations. The methoxyl value of 8% for protokosin is high for one methoxyl group (6·8%), but some isomerisation to kosin may occur in the course of the determination; furthermore the considerably different figure of 10·4% was found by other analysts. Recalculation of the *C*-Me figures (Hems and Todd, *loc. cit.*) for the C_{25}-formula gives maximum values of just over 3 for β-kosin and 3·7 for protokosin, indicating that these compounds may well contain the 4 and 5 *C*-Me groups to be expected on the assumption that an *iso*butyryl group is estimated as one *C*-Me. The fact that the figure for protokosin is significantly higher than for β-kosin supports the postulated migration from carbon to oxygen during the formation of the latter.

The fully methylated kosins, obtained by exhaustive methylation with methyl sulphate

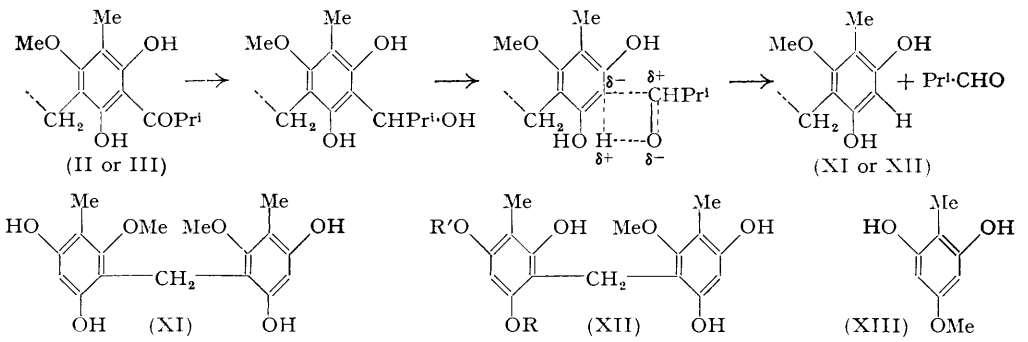
and sodium hydroxide, have now been shown to be identical by means of their infra-red spectra. The difference between the kosins therefore results from differing positions of the methylated hydroxyl groups; this is further confirmed by the identity of the fully methylated reduction products described below.

The infra-red spectra of protokosin and the kosins (see Experimental section) show a strong likeness, particularly the latter compounds, and possess bands due to (probably) hydrogen-bonded carbonyl (6·2 μ), hydrogen-bonded hydroxyl (general absorption in the region 3·3 μ), and normal hydroyl groups (3 μ). This is the first direct demonstration that the substances do, in fact, contain carbonyl groups, since no derivatives have so far been obtained which can be definitely identified as derived from the intact molecule. In the case of the fully methylated kosins the carbonyl band has shifted to the normal value of 5·85 μ. In order to be certain that the postulated non-aromatic ring in (I) can be reconciled with the infra-red spectrum, that of albaspidin (IX), which contains a similar system, has been examined. The carbonyl band here at 6·13 μ is also at a longer wave-length than usual, and there is no sign of a normal band at *ca.* 5·9 μ. The presence of two OH-bands (?) in protokosin (2·96, 3·23 μ) is somewhat puzzling, and they are not found in any of the other compounds. The 3·23 μ band may be due to a hydrogen-bonded hydroxyl group.

From the fact that the kosins fail to react with diazoaminobenzene, one and possibly both methoxyls are probably *ortho* to the methylene group (Boehm, *Annalen*, 1903, **329**, 301). In order to obtain more information it would be necessary either to synthesise the compounds, which should be possible by standard methods (*e.g.*, McGookin, Robertson, and Simpson, *J.*, 1951, 2021), or else to convert them into more readily synthesised compounds. Sodium amalgam seemed a possible reagent for this purpose, because its action might be expected to lead to fission of the molecule, or removal of the *iso*butyryl groups, or both. In fact, the action of 3% sodium amalgam on a cold alkaline solution, followed by heating, resulted in the formation of *iso*butaldehyde and methylenebismethylphloroglucinol derivatives in fair yield. The fission is evidently a reverse aldol condensation, as shown below, and depends on the ready production of an anion from the phloroglucinol nucleus. Other examples of the formation of such anions are the zinc dust fissions extensively used by Boehm, and the disproportionation reactions observed by McGookin *et al.* (*loc. cit.*).

The reduction products obtained were crystalline solids, $C_{17}H_{20}O_6$, α-, m. p. 231—232°, from α-kosin and β-, m. p. 236—237°, from β-kosin, which showed a small depression of melting point on admixture. The α-compound closely resembled (XI) which has been synthesised by Boehm (*Annalen*, 1903, **329**, 283) who gives m. p. 228—229°. The α-compound gave no indophenol reaction with 2 : 6-dichloroquinonechloroimide, whereas the β-compound gave an immediate dark blue colour. The former, therefore, has no unsubstituted position *para* to a hydroxyl group, while the latter has such a free position (Gibbs, *J. Biol. Chem.*, 1927, **72**, 649). The only formula for the α-kosin product which fits this fact is (XI), and since from its origin the β-kosin product presumably has one ring identical with those in (XI) it must be either (XII; R = H, R′ = Me) or (XII; R = Me, R′ = H). Of these possibilities only the latter provides a ready explanation of its formation from protokosin (see below). The reported formation from α-kosin of only the one phloroglucinol methyl ether (VI; R = Me) (Lobeck, *loc. cit.*) is additional evidence in favour of the symmetrical formula. The compound (VI; R = Et) has $\lambda_{max.}$ 274 mμ, and (XIII) has $\lambda_{max.}$ 270·5 mμ (Mulholland, *loc. cit.*), so it was hoped that the unsymmetrical compound would show two distinguishable maxima in this region. In fact, however, the α-compound has three (271·5, 275·5, 279·3 mμ) and the β-compound two (271, 275 mμ); these absorptions are in any case quite in accord with the postulated structures and 1 : 3 : 5-trimethylphloroglucinol has two maxima (271, 274 mμ). The infra-red spectra of the kosin reduction products are very similar, but not identical. They differ from the kosins, as expected, in possessing no carbonyl band and in having a much enhanced hydroxyl band. Complete methylation of the two compounds gave the same derivative, m. p. 114—115°, which was shown to be 2 : 4 : 6 : 2′ : 4′ : 6′-hexamethoxy-3 : 3′-dimethyldiphenylmethane by synthesis, thus confirming the correctness of the skeleton assigned to the kosins. Syntheses of the kosins themselves and their reduction products are in hand.

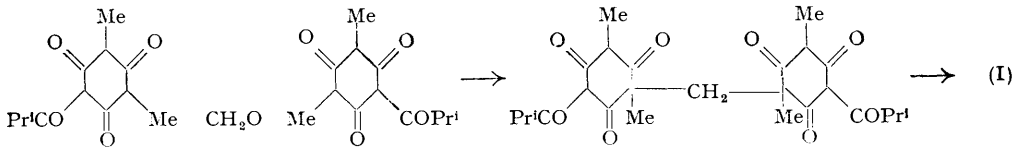
The disproportionation reactions observed by McGookin *et al.* (*loc. cit.*) raise the question whether the kosins may not in fact be symmetrical compounds produced by disproportionation of an unsymmetrical precursor formed from protokosin. However, there is no evidence of any equilibrium being set up because a third kosin has never been detected and both compounds are relatively stable in alkaline solution. The problem can only be resolved by synthesis, and in any case the general conclusions are not affected.



Formula (I) for protokosin explains its optical activity and the formation of the two kosins by migration of the angular methyl group in two possible directions, and is in accord with the absorption spectra. The unusual alkali-catalysed rearrangement is perhaps not too surprising in view of the already remarked anionoid character of the acylphloroglucinol nucleus; the driving force is presumably the tendency to form a fully aromatic system. Aromatisation by the more usual acid-catalysed type of process involving migration to carbon is impossible because of the substitution. The resemblance to the aspidin–ψ-aspidin change (Boehm, *Annalen*, 1903, **329**, 321) is marked, and the latter compound bears a close resemblance to the kosins from one of which it may differ, on present views, merely by the presence of *n*-butyryl instead of *iso*butyryl residues. Whether ψ-aspidin is in fact a single substance is not clear, but kosin was originally considered to be such before close investigation. The recorded melting point seems to vary considerably, and is lower than that of methylenebisaspidinol, with which it could be identical. Aspidin is unfortunately rather inaccessible, and we have been unable to obtain a sample of it for investigation.

The main evidence which appears at first sight to be incompatible with structure (I) is the formation of 1 : 3 : 5-trimethylphloroglucinol in the zinc dust–alkali reduction of protokosin. However, the equilibrium $R{\cdot}CH_2{\cdot}R' + H_2O \overset{OH^-}{\rightleftharpoons} R{\cdot}CH_2{\cdot}OH + R'H$ may be set up in alkaline solution (cf. Birch, *J.*, 1951, 3026; McGookin *et al.*, *loc. cit.*) or, alternatively migration of $R{\cdot}CH_2$ may occur to some extent forming $R{\cdot}CH_2{\cdot}OR''$ in a manner analogous to the migration of the methyl group which gives rise to the kosins. Either this product or $R{\cdot}CH_2{\cdot}OH$ could then react in the alkaline medium with reduction products such as dimethylphloroglucinol, and reduction of the resulting condensation product would yield trimethylphloroglucinol. It is worthy of note that in the reduction of 20 g. of protokosin, Hems and Todd (*loc. cit.*) were only able to account for a fraction of the starting material as isolated products, these being 6 g. of mixed α- and β-kosin, 1·5 g. of 1 : 3 : 5-trimethylphloroglucinol, and a small amount of *iso*butyric acid. We have, unfortunately, been unable to study this reaction further owing to the very small amount of protokosin at present available. It is pertinent that Boehm (*Annalen*, 1903, **329**, 269) found that reduction of methylenebisphloroglucinol itself gave some trimethylphloroglucinol. If the mechanism here suggested for the formation of trimethylphloroglucinol could be substantiated it would destroy the only evidence in favour of the four-membered ring (cf., *e.g.*, IV) postulated by Boehm to occur in a number of compounds from *Aspidium filix mas*, such as flavaspidic and filicic acids, and other structures which do not contain the carbon skeleton of trimethylphloroglucinol could be considered for these compounds. It is of some interest that structure (I) for protokosin could theoretically arise by the

condensation of two symmetrical units with formaldehyde, followed by a kosin-type migration of one methyl group.



Experimental

M. p.s are uncorrected.

Reduction of the Kosins.—*β-Kosin.* β-Kosin (1·00 g.) in sodium hydroxide solution (20 c.c.; 10%) was stirred with 3% sodium amalgam (30 g.) during 4 hours. The aqueous solution was then separated from the liquid amalgam, which was washed with water (20 c.c.). The combined aqueous solutions were distilled rapidly until the distillate failed to react with Brady's reagent (about 10 minutes). The 2 : 4-dinitrophenylhydrazone obtained from the distillate was crystallised three times from ethanol (Found: C, 48·0; H, 4·5. Calc. for $C_{10}H_{12}O_4N_4$: C, 47·6; H, 4·8%). It had m. p. 172—174°, undepressed by an authentic specimen of the derivative of *iso*butaldehyde, m. p. 176°. The aqueous residue from the distillation was acidified, and the curdy precipitate taken up in ether (30 c.c.) and extracted successively with saturated sodium hydrogen carbonate solution, N-sodium carbonate (3 × 10 c.c.), and N-sodium hydroxide (10 c.c.). All the extracts in acidification gave some precipitate, but only that from the sodium carbonate could be crystallised. Recrystallisation of the 4 : 6 : 2′ : 4′-*tetrahydroxy*-2 : 6′-*dimethoxy*-3 : 3′-*dimethyldiphenylmethane* (?) from acetone–benzene or aqueous methanol gave colourless needles, m. p. 236—237° (decomp. after softening at *ca.* 230°) (280 mg.) (Found: C, 63·8; H, 6·4. $C_{17}H_{20}O_6$ requires C, 63·75; H, 6·25%). The absorption in the ultra-violet showed the following peaks: maxima at 275 (ε, 1750) and 271 mμ (ε, 1800), and minima at 274 (ε, 1740) and 256 mμ (ε, 1130). In a phosphate buffer at pH 9 it gave an immediate deep blue colour with 2 : 6-dichloroquinonechloroimide.

α-Kosin. α-Kosin (80 mg.), similarly reduced with the same proportions of reagents, gave the same 2 : 4-dinitrophenylhydrazone, m. p. 172—174°, and 4 : 6 : 4′ : 6′-*tetrahydroxy*-2 : 2′-*dimethoxy*-3 : 3′-*dimethyldiphenylmethane*, m. p. 230—231° (decomp.) (35 mg.) (Found: C, 63·8; H, 6·35. Calc. for $C_{17}H_{20}O_6$: C, 63·75; H, 6·25%). Ultra-violet absorption: maxima at 279·3 (ε, 1570), 275·5 (ε, 1730), and 271·5 mμ (ε, 1780); minima at 278·5 (ε, 1560), 273·8 (ε, 1790), and 256·5 mμ (ε, 1100). In a phosphate buffer at pH 9 it gave with 2 : 6-dichloroquinone-chloroimide only a faint pink colour which slowly became green. Methylation of this reduction product, and of that obtained above from β-kosin, gave the same compound as colourless prisms [from ether–light petroleum (b. p. 40—60°)], m. p. 114—115° (Found: C, 67·0; H, 7·3. $C_{21}H_{28}O_6$ requires C, 67·0; H, 7·4%). This was shown to be 2 : 4 : 6 : 2′ : 4′ : 6′-*hexamethoxy*-3 : 3′-*dimethyldiphenylmethane* by the following synthesis. Methylphloroglucinol trimethyl ether (700 mg.) was added to a solution of formaldehyde (40%; 0·25 c.c.) and sulphuric acid (1 c.c.) in methanol (10 c.c.), and the mixture refluxed for 10 minutes. Water (20 c.c.) was then added, the mixture extracted with ether, and the ether washed with water, dried, and evaporated. Addition of light petroleum (b. p. 40—60°) (3 c.c.) to the residue produced a crystalline precipitate which was recrystallised from ether–light petroleum (b. p. 40—60°), to give colourless prisms, m. p. 114—115°, undepressed by the compound above.

Methylation of the Kosins.—The kosins were difficult to methylate completely, but the alternate addition of methyl sulphate and N-sodium hydroxide on the steam-bath under nitrogen was carried out until a test-portion of the gummy product gave no colour with alcoholic ferric chloride. The alkali-insoluble gum was then passed in light petroleum (b. p. 40—60°) through a column of alumina, the pale yellow, apparently uniform, band being eluted with light petroleum (b. p. 40—60°). The 5 : 5′-*di*iso*butyryl*-2 : 4 : 6 : 2′ : 4′ : 6′-*hexamethoxy*-3 : 3′-*dimethyldiphenylmethane* was an almost colourless gum which could not be crystallised (Found: C, 67·8; H, 8·1. $C_{29}H_{40}O_8$ requires C, 67·4; H, 7·9%). The infra-red spectra are given below.

Methylenebisbaeckeol.—A mixture of baeckeol (400 mg.) (Hems and Todd, *J.*, 1940, 1208), paraformaldehyde (60 mg.), and a mixture of sulphuric acid (0·5 c.c.), water (0·5 c.c.), and methanol (5 c.c.) were shaken for 24 hours and then heated on the steam-bath for 30 minutes. The yellow needles which separated on cooling were recrystallised from acetone–methanol, to

give *methylenebisbaeckeol*, m. p. 177—178° (Found: C, 66·7; H, 7·0. $C_{27}H_{36}O_8$ requires C, 66·4; H, 7·3%). It gave a dark green colour with alcoholic ferric chloride.

Infra-red Spectra.—The spectra were determined for Nujol mulls in a Perkin-Elmer Model 21 double-beam instrument; the Nujol bands are omitted from the results. s = strong, m = medium, w = weak, s-b = side-band (λ in μ).

Protokosin. 2·96w, 3·23w, 6·20s, 6·45s-b, 7·11s, 7·38s-b, 7·85w, 8·15w, 8·45m, 8·67m, 8·99m, 9·12m, 9·65w, 10·01w, 10·33w, 10·63w, 10·80w, 11·02m, 13·13w, 13·77w.

α-*Kosin.* 3·13m, 6·20s, 7·10m, 7·40s-b, 7·67w, 7·87s, 8·43m, 8·63w, 9·00s, 9·35w, 9·84w, 10·10m, 10·77w, 12·3w.

β-*Kosin.* 3·10m, 6·24s, 7·13m, 7·34m, 7·63w, 7·89m, 8·45s, 9·02s, 9·34w, 9·85m, 10·05m, 10·80w, 11·2w, 12·1w.

α-*Kosin reduction product.* 3·0s, 6·11m, 6·17m, 6·60m, 7·31m, 7·66m, 7·90w, 8·16w, 8·28w, 8·80s, 9·30s, 9·90w, 11·19w, 11·55w, 12·51s, 12·85w, 13·10w, 13·90w.

β-*Kosin reduction product.* 3·0m, 6·13w, 6·20w, 6·62w, 7·34s-b, 7·7w, 7·90w, 8·18w, 8·30w, 8·82s, 9·33s, 9·90w, 12·53m.

Flavaspidic acid. 3·16w, 6·10s, 6·20s, 7·75m, 7·9m, 8·38s, 8·65m, 9·74w, 10·8w, 11·14w, 11·85w, 12·45w, 12·94w, 13·85w.

Albaspidin. 2·97w, 6·13s, 6·38s, 7·54w, 7·77m, 8·14w, 8·33m, 9·60w, 10·44w, 10·66w, 11·4w, 11·67w, 12·26w, 12·45w, 12·66w, 13·57w.

Methylated α- and β-kosin (β- *in brackets*). 5·87(5·89)s, 6·31(6·32)s, 6·87(6·87)s, 7·11(7·11)s, 7·24(7·24)m, 7·57(7·57)m, 7·9(7·87)w, 8·36(8·37)s, 9·03(9·03)s, 9·50(9·50)m, 9·90(9·90)s, 10·17(10·17)s-b, 10·53(10·53)w, 10·77(10·80)w, 10·94(10·96)m, 11·23(11·23)w, 11·55(11·55)w, —(11·85)w, 12·40(12·40)w.

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