125. *Anthelmintics: Kousso. Part I. Protokosin.*

By B. A. Hems and A. R. Todd.

The dried female flowers of *Hagenia abyssinica* (= *Brayera anthelmintica*) have long been used as an anthelminthic drug under the name " kousso " or " koso," particularly in North Africa and the Near East; in Europe it has been largely displaced by extracts of the male fern (*Filix mas*). Investigations of kousso were made by numerous workers from 1839 onwards, but the early literature is in a very confused state; a useful summary of the early work is given by Kondakow (*Arch. Pharm.*, 1899, **237**, 481). The first definite crystalline product from kousso appears to have been the *kosin*, m. p. 142°, prepared by Messrs. E. Merck by an extraction process involving treatment of the flowers with hot milk of lime and subsequent extraction with alcohol. This preparation was investigated by Flückiger and Buri (*Arch. Pharm.*, 1874, **5**, 205), who ascribed to it a formula $C_{31}H_{38}O_{10}$. Leichsenring (*ibid.*, 1894, **232**, 50) isolated from an ethereal extract of kousso, protokosin, a colourless crystalline compound with no vermicidal properties, and kosotoxin, an amorphous, highly toxic substance: to them he allotted the formulæ $C_{29}H_{38}O_9$ and $C_{26}H_{34}O_{10}$ respectively. By treatment of protokosin with hot baryta he obtained a product identical with Merck's kosin, for which he proposed a formula $C_{23}H_{30}O_7$. Lobeck (*Arch. Pharm.*, 1901, **239**, 672), using a modified form of the old alkaline extraction process, isolated kosin together with small quantities of protokosin and a third substance, kosidin, having very similar properties. His analytical data for kosin and protokosin agreed with those of Leichsenring (*loc. cit.*), but he was able to show that the so-called kosin was really a mixture of two isomers, α- and β-kosin. Apart from the fact that both kosin and kosotoxin gave on akali fusion *iso*butyric acid and traces of substances resembling phloroglucinol derivatives, nothing further appears to be known regarding the nature of any of the above substances.

From an ethereal extract of kousso we have isolated, in a yield of 0·4% and by a much simpler process than that of Leichsenring (*loc. cit.*), a substance having the recorded properties of protokosin. A search was made for Lobeck's kosidin, but none could be isolated;

from his description of its preparation and properties we are of the opinion that his product was mainly impure protokosin. The amorphous material to which Leichsenring gave the name kosotoxin was also obtained, but has not yet been thoroughly investigated.

Analysis and molecular-weight determinations indicate for protokosin a formula $C_{22}H_{28}O_7$ rather than that proposed by Leichsenring (*loc. cit.*); it contains one methoxy-group, and side-chain methyl estimations (Kuhn–Roth) indicate the probable presence of four $CH_3{\cdot}C$ groups. Protokosin has phenolic properties, but, like earlier workers, we are unable to prepare any crystalline derivatives, though an amorphous acetate which seemed to contain three acetyl groups was obtained; in solution it is dextrorotatory. Alkaline degradation of protokosin was found to be more satisfactory with sodium hydroxide than with barium hydroxide which was used by Leichsenring; on heating for 5 minutes with 20% aqueous sodium hydroxide in presence of zinc dust it yielded a fatty acid, and two other crystalline products, one lemon-yellow and one colourless.

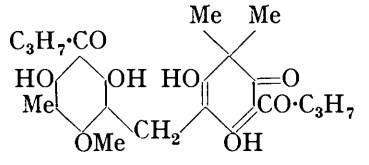
The colourless product was identified by comparison with synthetic material as *C*-trimethylphloroglucinol and the relatively mild conditions of reaction justify the assumption that in its formation no demethylation has occurred. The pale yellow product was identical with Merck's kosin, of which a small sample was available for comparison through the kindness of Messrs. E. Merck, Darmstadt, to whom our thanks are due. By fractional crystallisation we were able to separate this material into two distinct compounds, α-kosin, m. p. 158° (yellow needles), and β-kosin, m. p. 120° (yellow prisms), the former being present in much larger quantity; these compounds are presumably identical with those obtained by Lobeck (*loc. cit.*) from Merck's kosin. Analysis and molecular-weight determinations showed that α- and β-kosin are isomeric with each other and with protokosin; they differ from the latter compound (1MeO) in that both contain two methoxy-groups and they are optically inactive. They are phenolic in nature, and form crystalline triacetyl derivatives. Accurate methoxyl determination is difficult with these compounds, repeated analyses failing to give satisfactory values; this difficulty has also been encountered by Boehm in his studies of compounds from *Aspidium filix mas* (cf. *Annalen*, 1901, **318**, 230). α- and β-Kosin are very resistant to further breakdown by alkali; after boiling even with 80% sodium hydroxide, most of the starting material was recovered unchanged; from the part attacked, no identifiable products could be isolated.

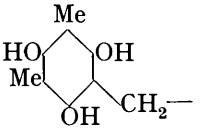
The fatty acid obtained in the alkaline treatment of protokosin is also produced by the action of sulphuric acid, and was reported by Leichsenring, on scanty evidence, to be *iso*butyric acid. This we have confirmed, the acid being identified as its *p*-phenylphenacyl ester and its *p*-toluidide. The amount of *iso*butyric acid liberated on heating protokosin with sulphuric acid at 160°, or with alkali at 100°, was determined; the figure obtained corresponded approximately to the presence of one *iso*butyryl group in the original substance.

When fused with alkali, protokosin gave, in addition to amorphous products, a small yield of a crystalline substance, identified as *C*-monomethylphloroglucinol by comparison with a synthetic specimen. A variety of oxidative and other degradations were carried out on protokosin, but all gave negative results.

The properties and reactions of protokosin are reminiscent of those shown by compounds isolated from the anthelmintic *Aspidium filix mas* (cf. Boehm, *loc. cit.*). The remarkable conversion of protokosin into α- and β-kosin under the influence of alkali has a parallel in the behaviour of aspidin (I), which under similar conditions yields an isomeric yellow alkali-stable compound containing an additional methoxy-group (Boehm, *Annalen*, 1903, **329**, 321). The mechanism of this reaction is not clear; it apparently involves the migration of a methyl group from carbon to oxygen. Furthermore many compounds of the *Aspidium filix mas* group contain a butyryl group removable by acid or alkali, and give phloroglucinol derivatives on alkali treatment.

(I.)



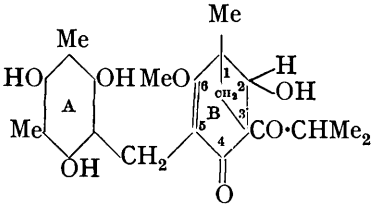


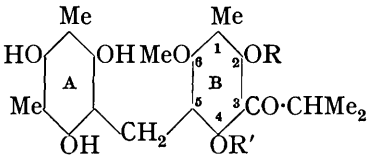
(II.)

It seems reasonable to assume that protokosin is somewhat similarly constituted to the *Aspidium filix mas* products, *i.e.*, that it is a derivative of methylenebisphloroglucinol.

The isolation of *C*-trimethylphloroglucinol as described above fixes for one ring the structure (II); for the remainder of the molecule the evidence is not decisive, but sufficient is available to permit of a suggestion as to its probable nature. This portion of the molecule must accommodate the methoxy-group and the *iso*butyryl side chain, and at the same time explain its optical activity and allow for the conversion into the isomeric inactive α- and β-kosins, which are both phenolic and more stable than protokosin. A possible formula for protokosin which fulfils these requirements is (III); a definite formula can only be assigned to it when further evidence becomes available.

(III.)





(IV.)

The methylene bridge in ring B is preferred to a *gem*-dimethyl group, since oxidation of protokosin, by the method employed by Boehm (*Annalen*, 1901, **318**, 230) in the case of filix acid, yielded no *gem*-dimethylphloroglucinol derivatives, nor could dimethylmalonic acid be obtained by potassium permanganate oxidation under conditions known to permit of its isolation. Protokosin does not react with the usual carbonyl reagents, possibly for steric reasons, and it gives no condensation product with diazoaminobenzene; the latter fact indicates that, if it is a methylenebisphloroglucinol derivative, it bears a methoxy-group in an ortho-position to the point of attachment of the methylene bridge between the two nuclei and contains no free methin group (Boehm, *Annalen*, 1903, **329**, 269). The position of the methoxy-group also explains the failure to obtain a xanthene derivative on warming with a solution of hydriodic acid in glacial acetic acid.

On the basis of formula (III) the two kosins could be represented by (IV; R = H; R′ = Me) and (IV; R = Me; R′ = H), the position of the second methoxy-group being deduced as follows. Protokosin apparently yields only a triacetate, and it seems most likely on steric grounds that the hydroxyl in position 2 is the one unaffected by acetylation. Analogies are found for this in *Aspidium filix mas* products, *e.g.*, aspidin (I) gives only a di- and not a tetra-acetate (Boehm, *loc. cit.*). Both α- and β-kosin also give triacetates. It is clear that when ring B in protokosin is aromaticised by rupture of the bridge between C_1 and C_3 a new hydroxy-group appears at C_4; this should offer the same resistance to acylation as the C_2 hydroxyl, the steric hindrance being similar in both cases. If, then, the new methoxy-group in each of the kosins is situated in ring B, on acetylating them only the three hydroxyls in ring A will be affected; it is difficult to find any other formulation which explains the production of triacetates.

It is not possible to draw any conclusions from the production of *C*-monomethylphloroglucinol on fusing protokosin with alkali, since the treatment was so drastic that its formation by further degradation of *C*-trimethylphloroglucinol cannot be excluded.

Experimental.

Isolation of Protokosin.—Dried commercial kousso (25 kg.) was exhaustively extracted with ether by percolation at room temperature, and the extract concentrated to a dark green syrup (*ca.* 2·5 l.). This syrup was extracted in portions of 500 g. with boiling light petroleum (2 l., b. p. 40—60°), and the extracts decanted off and evaporated. The residue, dissolved in hot alcohol (1 l.) and allowed to stand for 2 days, deposited a considerable amount of waxy material, which was filtered off; after concentration to about 300 c.c. the filtrate slowly deposited *protokosin*, which was collected at intervals of 2—3 days during 2 weeks. On prolonged standing, further small amounts of the same product separated. Total yield, 100 g.; m. p. 179°.

The crude product, which had a slightly greenish colour, crystallised from chloroform–

alcohol in colourless needles, m. p. 182°, not raised by further crystallisation [Found: C, 65·4, 65·2, 65·4; H, 7·0, 7·0, 7·0; MeO, 8·1, 8·0, 8·1; *M* (Rast), 412, 396, 399. $C_{21}H_{25}O_6(OMe)$ requires C, 65·4; H, 6·9; MeO, 7·6%; *M*, 404].

Oxidation with chromic acid (Kuhn–Roth) gave 3·0, 3·3 mols. of acetic acid, corresponding to the presence of four side-chain methyl groups. Hydroxyl estimations (Zerewitinoff) gave 11·6, 11·7% OH (calc. for three OH, 12·6%) (cf. α- and β-kosin below).

Protokosin has the properties given by Leichsenring (*loc. cit.*). It is insoluble in water, dissolves with difficulty in alcohol and light petroleum, and is readily soluble in ether, acetone, ethyl acetate, and chloroform. It is insoluble in cold sodium carbonate solution, but dissolves slowly in sodium hydroxide to a yellow solution. Its solution in concentrated sulphuric acid, at first pale green, becomes deep red on warming, and alcoholic solutions give with ferric chloride a red-brown colour. It decolorises potassium permanganate rather slowly and does not react with 2 : 4-dinitrophenylhydrazine. In chloroform solution (*c*, 10%) it has $[\alpha]_D$ + 8·0°. No crystalline acyl or methoxy-derivatives could be prepared. No xanthene derivative was formed on heating with a solution of hydriodic acid in acetic acid.

Acetylation of Protokosin.—Protokosin (500 mg.) was dissolved in pyridine (5 c.c.), and acetic anhydride (5 c.c.) added; after standing in the cold for 36 hours, the solution was poured into water. A friable solid separated, m. p. 90—100°. It could not be purified (Found by hydrolysis with hot 2% potassium hydroxide: CH_3·CO, 22·4. Calc. for a triacetate: CH_3·CO, 23·8%).

Kosidin and Kosotoxin.—Efforts to isolate kosidin (Lobeck, *loc. cit.*) from the light petroleum insoluble fraction of the kousso extract were unsuccessful; no evidence for the presence of any crystalline material other than a little protokosin could be obtained. In view of this result and of the close similarity between the recorded properties of kosidin and protokosin, we incline to the view that the so-called kosidin of Lobeck was simply impure protokosin. The kosotoxin obtained from the alcoholic mother-liquors in the extraction process has not yet been submitted to detailed investigation.

Alkali Treatment of Protokosin.—A mixture of protokosin (20 g.), zinc dust (10 g.), and aqueous sodium hydroxide (200 c.c. of 20%) was heated to boiling for 5 minutes, cooled, and filtered from zinc. The yellow filtrate was acidified and extracted several times with ether, the extract being washed with sodium bicarbonate solution. After drying over sodium sulphate, the combined ethereal extracts were evaporated, and the residue dissolved in a small amount of methyl alcohol. On cooling to — 15°, "kosin" (*ca.* 6 g.) separated.

After separation of the kosin, the solution was evaporated, and the residue boiled with benzene until the insoluble portion became powdery; the insoluble material crystallised from water in colourless needles (1·5 g.), m. p. 184° (Found: C, 64·8; H, 7·0. Calc. for $C_9H_{12}O_3$: C, 64·3; H, 7·1%). That this product is *C*-trimethylphloroglucinol was established by comparison with a specimen of that substance (m. p. 184°) prepared by the method of Weidel (*Monatsh.*, 1898, **19**, 250); the reactions were identical and a mixed m. p. showed no depression.

The sodium bicarbonate washings from the above experiment were acidified, combined with the original aqueous acid liquors, and steam-distilled. The distillate, which smelt strongly of *iso*butyric acid, was extracted with ether, the extract dried, and the ether distilled off. From the acidic residue the *p*-phenylphenacyl ester was prepared by the method of Drake and Bronitsky (*J. Amer. Chem. Soc.*, 1930, **52**, 3715); it had m. p. 72°, not depressed by the ester (m. p. 72°) prepared from authentic *iso*butyric acid. For further confirmation a *p*-toluidide was prepared. Recrystallised from light petroleum, this had m. p. 104°, alone or mixed with *iso*butyro-*p*-toluidide (m. p. 104°). No trace of *n*-butyric or other acids was detected.

Fractionation of Kosin.—Repeated crystallisation of crude kosin (6 g.) from methyl alcohol yielded two yellow substances, the less soluble α-kosin (*ca.* 4·5 g.) crystallising in needles and the much more soluble β-kosin (*ca.* 1 g.) forming prisms.

α-Kosin forms pale yellow needles, m. p. 158°, when pure; the ordinary product has m. p. 152°, which can only be further raised with great difficulty [Found: C, 65·3, 65·1; H, 7·0, 6·8; MeO, 13·5, 13·2; *M*(Rast), 393. $C_{20}H_{22}O_5(OMe)_2$ requires C, 65·4; H, 6·9; MeO, 15·3%; *M*, 404]. α-Kosin is optically inactive. Hydroxyl determinations (Zerewitinoff) gave rather unsatisfactory values approximating to three hydroxy-groups. A series of degradative experiments gave no crystallisable products.

Triacetyl-α-kosin.—α-Kosin (150 mg.) was heated on the water-bath for 2 hours with acetic anhydride (2 c.c.) and fused sodium acetate (200 mg.). The solution was poured into water and extracted with ether, and the extract washed with sodium bicarbonate solution, dried, and evaporated. The residue crystallised from light petroleum in colourless needles, m. p. 123°

[Found: C, 63·8; H, 6·5; CH_3·CO, 24·5. $C_{22}H_{25}O_4(O{\cdot}CO{\cdot}CH_3)_3$ requires C, 63·6; H, 6·4; CH_3·CO, 23·8%].

β-*Kosin* forms very pale yellow prisms, m. p. 120° [Found: C, 65·3; H, 7·0; MeO, 13·4; *M* (Rast), 356. $C_{20}H_{22}O_5(OMe)_2$ requires C, 65·4; H, 6·9; MeO, 15·3%; *M*, 404]. It is optically inactive and no crystalline degradation products could be obtained from it. Hydroxyl estimations (Zerewitinoff) gave values approximating to three hydroxy-groups at 20° and four at 95°.

Triacetyl-β-kosin.—β-Kosin (100 mg.) was acetylated in the manner described above for α-kosin. The product formed colourless needles, m. p. 155° [Found: C, 63·8; H, 6·8; CH_3·CO, 24·5. $C_{22}H_{25}O_4(O{\cdot}CO{\cdot}CH_3)_3$ requires C, 63·6; H, 6·4; CH_3·CO, 23·8%].

Quantitative Estimation of iso*Butyric Acid formed from Protokosin.*—(a) Protokosin (210 mg.) was refluxed for 20 minutes with 10% sodium hydroxide solution (20 c.c.), cooled, acidified with sulphuric acid, and steam-distilled. The distillate was titrated against *N*/10-sodium hydroxide (Found: C_3H_7·CO, 15·1%).

(b) Protokosin (202 mg.) was heated in a sealed tube at 160° for 6 hours with 15% sulphuric acid (6 c.c.). The product was steam-distilled, and the distillate titrated (Found: C_3H_7·CO, 15·1%).

The calculated value for one *iso*butyryl group in protokosin is C_3H_7·CO, 17·6%.

Alkali Fusion of Protokosin.—An intimate mixture of protokosin (5 g.), potassium hydroxide (10 g.), and a little water was heated for 15—20 minutes at 300°. The mixture reddened, frothed, and finally became black. The cooled melt was dissolved in water, acidified, and steam-distilled, and the residue extracted repeatedly with ether. After being decolorised with charcoal, the ethereal solution was dried and evaporated. On recrystallisation of the residue from water (charcoal), a small amount of a substance having the reactions of a phloroglucinol derivative was obtained. After several crystallisations it had m. p. 208°, undepressed by admixture with *C*-monomethylphloroglucinol (m. p. 208°); both substances had identical properties and reactions. No other crystalline product could be isolated.

We are indebted to Prof. A. Robertson for a sample of synthetic *C*-monomethylphloroglucinol.

In connection with the identification of the above product *C*-monomethylphloroglucinol was synthesised, the method described by Curd and Robertson (J., 1933, 437) being followed. We found, however, that the following modified preparation of ethyl methylphloroglucinoldicarboxylate led to considerably improved yields: To potassium ethoxide (from 1·9 g.; 2 atoms) in ether is added acetonedicarboxylic ester (5 g.), followed by ethyl methylmalonate (4·6 g.). The mixture is thoroughly shaken, and the ether cautiously evaporated. The residue is heated at 160° for 1 hour, and the solid cake cooled, dissolved in water (100 c.c.), extracted with ether, and saturated with carbon dioxide. In the course of ½ hour yellow crystals of ethyl methylphloroglucinoldicarboxylate separate. Recrystallised from alcohol, it has m. p. 90° (yield, 2 g.).

Other Experiments with Protokosin.—All known methods of methylation were applied, but only resins were obtained; similar failures were encountered on acylation, treatment with trityl chloride and phenyl *iso*cyanate. No reaction could be detected with the usual carbonyl reagents.

Oxidation experiments with chromic acid, selenium dioxide, and nitric acid gave only traces of lower fatty acids, and similar oxidation in neutral or alkaline solution with various amounts of potassium permanganate gave identical results; no oxalic acid was produced.

Catalytic hydrogenation in various solvents gave no crystalline product, although about 1 mol. H_2 seemed to be taken up; similar results were got with other reducing agents, *e.g.*, zinc; hydriodic acid and red phosphorus. Distillation with zinc dust gave only traces of a sharp-smelling oil, and no results were obtained from heating protokosin or its hydriodic acid reduction product with selenium. Numerous variations in the conditions of alkaline degradation were tried, but no products other than those above described could be isolated.

A similar lack of success attended degradation experiments of various kinds on α- and β-kosin.

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