36. *Afzelin (Kæmpferol-3-rhamnoside), a New Glycoside isolated from Doussié.*

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The principal constituent of a powdery material occluded in a sample of doussié, a timber derived from trees of the genus *Afzelia*, is *kæmpferol-3-rhamnoside*, a new glycoside to which the name *afzelin* has been given.

The conversion of a log of doussié, the timber of various species of *Afzelia*, revealed a " shake " filled with a pale brownish-yellow powder. Part of the log including the shake was submitted to the Director of the Forest Products Research Laboratory, Department of Scientific and Industrial Research, and we are indebted to Mr. W. G. Campbell of that Laboratory for a supply of the substance and for the following note on its occurrence. " Type specimens of the wood of *Afzelia* spp. commonly show a yellow deposit in some of the pores. The large deposit in the specimen under consideration probably arose as a result of the evaporation of water from tree sap which had oozed into a large fissure or ' shake.' Shakes are caused by various agencies such as, for example, internal stresses in the standing trees, damage by lightning or by impact with other trees during felling operations."

No clue as to the composition of the powder was to be found in the literature, which is without reference to any chemical investigation of the *Afzelia* genus, but from its solubility in water and the formation of a voluminous micro-crystalline precipitate on heating with aqueous acid, the presence of a glycoside was inferred. Further investigation revealed that apart from small quantities of two unidentified substances, the principal constituent is the formerly unknown *kæmpferol-3-rhamnoside*, for which we propose the name *afzelin*.

Several kæmpferol glycosides have been described which afford rhamnose on hydrolysis (see " The Chemistry of Natural Colouring Matters," Mayer-Cook, 1943, pp. 182, 183). One of these, robinin, is of interest in that on enzymic hydrolysis (Zemplen and Bognar, *Ber.*, 1941, **74**, 1783), kæmpferol-7-rhamnoside is obtained, apparently the only kæmpferol monorhamnoside hitherto definitely characterised. Of the others, in only three, *viz.*, kæmpferitrin, lespedin, and an unnamed compound found in the flowers of Australian acacia species by Petrie (*Biochem. J.*, 1924, **18**, 957), is rhamnose the sole glycosidic component. Although the nature of its hydrolysis products was clearly established, little more is known of the acacia colouring matter and nothing appears to have been done to characterise the glycoside or determine its composition. Lespedin, on the other hand, has been fully identified, and its constitution was shown by the usual chemical methods to be kæmpferol-3 : 7-dirhamnoside (Hattori and Hasegawa, *Proc. Imp. Acad. Japan*, 1940, **16**, 9; Hasegawa, *Acta Phytochim.*, 1940, **11**, 299). Kæmpferitrin, the yellow pigment of *Indigofera arrecta*, also gives two molecules of rhamnose when hydrolysed (A. G. Perkin, *J.*, 1907, **91**, 435), but there is no chemical evidence to show the

position of the sugar residues. On the supposition that those flavonol glycosides which differ appreciably in their ultra-violet absorption from the corresponding aglycones are 3-glycosides, Tasaki (*Acta Phytochim.*, 1925, **2**, 119, 129) has classified kæmpferitrin as a 3-bioside, but it is evident that the argument cannot be exclusive of a 3 : *x*-dimonoside structure. Moreover, the close resemblance observed by Tasaki between the spectra of quercetin and rutin, of which the latter was later shown by Attree and A. G. Perkin (*J.*, 1927, 234) to be quercetin-3-rutinoside, weakens any conclusions which may be drawn from these measurements.

Of the crude material collected from the afzelia "shake" some 20 g. were available for investigation. Hydrolysis of a portion with 2% sulphuric acid precipitated an aglycone which was undoubtedly kæmpferol (5 : 7 : 4′-trihydroxyflavonol), but a persistent impurity depressed its m. p. to 273—275°. The glycoside was therefore dissolved in aqueous ethanol, and tannins, etc., were precipitated with lead acetate. After removal of excess of lead as sulphide, the solution was evaporated, and the residue subjected to fractional crystallisation from water. The principal fraction consisted of the yellow crystalline afzelin, having the ferric chloride reaction and strong blue-green fluorescence in sulphuric acid characteristic of kæmpferol.

Hydrolysis of the purified glycoside liberated kæmpferol, m. p. 279°, identical with an authentic specimen of the flavonol kindly provided by Professor W. Bradley, Leeds University. From the filtrate neutralised with barium carbonate, phenylhydrazine acetate precipitated an osazone shown by direct comparison to be L-rhamnose osazone, m. p. 180—181°.

The position of the sugar residue was determined by methylation with excess of diazomethane and acid hydrolysis of the glassy product, whereby a crystalline *O*-trimethylkæmpferol was obtained having the properties of 5 : 7 : 4′-trimethoxyflavonol previously synthesised by Kostanecki, Lampe, and Tambor (*Ber.*, 1904, **37**, 2098). A determination of the ultra-violet absorption of the glycoside in methanol gave $\lambda\lambda_{max.}$ 2650, 3450 A.; $\lambda_{min.}$ 2810 A.; log $\varepsilon_{max.}$ 4·39, 4·12, and log $\varepsilon_{min.}$ 4·08, respectively, in fairly close agreement with $\lambda\lambda_{max.}$ 2675, 3700 A., and log $\varepsilon_{max.}$ 4·12, 4·28, for kæmpferol (Skarzynski, *Biochem. Z.*, 1939, **301**, 150). In its lack of a maximum at 3100 A. characteristic of kæmpferol and of flavonols in general unsubstituted in the 3-position (see Skarzynski, *loc. cit.*), afzelin thus conforms to Tasaki's classification.

From the solution obtained by extracting crude afzelin with a limited quantity of boiling water a colourless crystalline *compound*, $C_{15}H_{12}O_6$, was isolated. Its properties suggest that it has a tetrahydroxydihydroflavone structure, but the amount available did not admit of its full identification. The third substance encountered has not been obtained in a homogeneous condition.

Experimental.

Isolation of Afzelin.—The light powdery solid (8·6 g.) from the afzelin shake was dissolved in warm aqueous ethanol (250 c.c.; 40%). After being filtered from wood fragments, etc., the yellow solution was treated with aqueous lead acetate (100 c.c.; 10%) until no further immediate precipitation took place (the use of basic lead acetate caused precipitation of almost all the dissolved material). The product was then centrifuged, and the filtrate saturated with hydrogen sulphide to precipitate the residual lead, after which the solution was evaporated under reduced pressure to 150 c.c. A yellow precipitate (A) (1·95 g.), m. p. 155—160°, was formed after 24 hours at 0°. The filtrate from this was further concentrated (to 80 ml.) and when left at 0° for several days gave a second yellow precipitate (B) (4·1 g.), m. p. 158—165°. Little material remained in the solution, which was then discarded.

Product (A) was treated with boiling water (55 c.c.), thereby leaving a residue consisting of fairly pure afzelin. On cooling to room temperature, the filtrate deposited a very pale yellow solid (0·4 g.), later proved to be a new compound $C_{15}H_{12}O_6$ (*q.v.*). When this had been removed, a further deposition of afzelin (0·62 g.) occurred gradually at 0°. The precipitate was collected, and the filtrate, diluted to 150 c.c., was heated to boiling with product (B). Again the undissolved solid consisted of afzelin, and a further quantity (0·85 g.) crystallised at room temperature. Cooling to 0° caused the appearance of a solid (2·4 g.), which on recrystallisation was separated into afzelin (0·5 g.), and a yellow substance (1·9 g.), m. p. 208—212° (sintering at 150°); unlike the other two products isolated, it gave in ethanol solution a yellow ferric reaction. This impure product was not further examined.

The glycoside *afzelin* crystallised from water–ethanol (4 : 1) as a hydrate consisting of yellow prisms, m. p. 172—174° (effervescence) (Found: C, 54·7, 55·1; H, 5·3, 5·3. $C_{21}H_{20}O_{10},1\frac{1}{2}H_2O$ requires C, 54·9; H, 5·0. Found, after drying at 125—130° in a vacuum: C, 57·8, 57·3; H, 5·2, 5·1; loss, 6·2. $C_{21}H_{20}O_{10}$ requires C, 58·3; H, 4·6; loss, 5·9%). A Zeisel determination showed the absence of methoxyl groups. It dissolved readily in alcohols and moderately in ether. Reduction with magnesium in alcoholic hydrochloric acid gave a red colour identical with that obtained from kæmpferol under the same conditions. Treatment with concentrated sulphuric acid changed the colour of the glycoside to orange, and it then dissolved to a yellow-green solution with an intense green-blue fluorescence; heating, which changed the colour to brown, finally caused charring. The addition of basic lead acetate to an aqueous alcoholic solution of the glycoside gave a yellow-green precipitate, but dilute lead acetate produced only an opalescence.

Hydrolysis of Afzelin.—The anhydrous glycoside (0·2002 g.) dissolved easily in hot aqueous 2% sulphuric acid (25 ml.). When the solution was refluxed for 1 hour a yellow precipitate was formed, which was collected next day, the filtrate and washings being retained.

The dried aglucone weighed 0·1324 g. (99·8% calculated on the basis of one rhamnose unit per molecule). It separated from glacial acetic acid in yellow prisms, m. p. 279°, alone or mixed with an authentic specimen of kæmpferol, m. p. 279° (Found, after drying at 110° in a vacuum: C, 62·7; H, 3·8. Calc. for $C_{15}H_{10}O_6$: C, 62·9; H, 3·5%).

The solution and washings from which the aglucone had been collected were heated on a steam-bath and neutralised with barium carbonate. After removal of the precipitated sulphate the liquid was evaporated, giving a brownish gum (0·0833 g.), which was dissolved in hot water (8 c.c.). A test portion of the solution reduced hot, but not cold, Fehling's solution. Aqueous phenylhydrazine acetate (0·4 c.c. of a mixture containing 10 c.c. of phenylhydrazine and 10 c.c. of acetic acid diluted to 25 ml. with water) was added, and after 10 minutes at 100° a precipitate formed (required for the formation of rhamnose osazone, 9 minutes). One hour later the mixture was cooled, and the osazone collected. Its crystalline form was identical with that of a specimen similarly prepared from L-rhamnose, and after crystallisation from ethanol it had m. p. and mixed m. p. 180—181° to a deep red liquid (Found: C, 62·3; H, 6·5. Calc. for $C_{18}H_{22}O_3N_4$: C, 63·1; H, 6·4%).

Methylation of Afzelin.—The glycoside (0·3 g.) in methanol solution (20 ml.) was repeatedly treated with ethereal diazomethane until the solution no longer gave a green colour with alcoholic ferric chloride. The pale brown gum obtained after removal of the solvent could not be induced to crystallise; it was therefore hydrolysed by boiling for 2 hours with 2% sulphuric acid (35 c.c.). Next day, the precipitate was collected, and after 3 crystallisations from aqueous ethanol it formed very pale yellow needles, m. p. 151° (Found: C, 62·6; H, 5·5. Calc. for $C_{18}H_{16}O_6,H_2O$: C, 62·4; H, 5·2. Found, after drying at 135° in a vacuum: C, 65·4; H, 5·1; loss, 5·6. Calc. for $C_{18}H_{16}O_6$: C, 65·85; H, 4·9; loss, 5·2%). The compound was evidently 3-hydroxy-5 : 7 : 4′-trimethoxyflavone, m. p. 151—152°, synthesised by Kostanecki, Lampe, and Tambor (*loc. cit.*), and gave identical reactions with dilute sodium hydroxide and concentrated sulphuric acid, and a dull reddish-black colour with ferric chloride in ethanol.

The Compound $C_{15}H_{12}O_6$.—The pale yellow solid (0·4 g.) obtained in the course of purifying afzelin dissolved completely in boiling water (33 c.c.) and on cooling crystallised in colourless needles. After two further crystallisations it melted at 225—227° to a deep-red liquid, after becoming yellow at 200° (Found: C, 55·6, 55·1; H, 5·0, 5·0. $C_{15}H_{12}O_6,2H_2O$ requires C, 55·6; H, 4·9. Found, after drying at 110° in vacuum: C, 62·2, 62·7; H, 4·3, 4·5; loss 10·7. $C_{15}H_{12}O_6$ requires C, 62·5; H, 4·2; loss, 11·1. Ultra-violet light absorption in methanol $\lambda_{max.}$ 2900 A., log $\varepsilon_{max.}$ = 4·24; $\lambda_{min.}$ 2510 A., log $\varepsilon_{min.}$ = 3·37; inflection at *ca.* 3270 A.). It contained no methoxyl groups. It dissolved very easily in ethanol, and the solution gave a dull red ferric colour. The *compound* was stable to boiling 2% aqueous sulphuric acid. It dissolved in the cold concentrated acid to a yellow solution devoid of fluorescence even under ultra-violet light. The colour was discharged by a trace of concentrated nitric acid; on heating it became red and finally darkened from charring. The compound reacted immediately with bromine in acetic acid, and dissolved in aqueous sodium hydrogen carbonate (3%) to a very pale yellow solution. Addition of sodium hydroxide deepened the colour, and the addition of acid regenerated the compound unchanged. It slowly reacted with alcoholic 2 : 4-dinitrophenylhydrazine to give a crystalline precipitate; reduction with magnesium in ethanolic hydrochloric acid gave a pink colour, lighter than that similarly obtained from kæmpferol or afzelin. A derivative separating from ethanol in colourless needles, m. p. 160—164°, was obtained by the action of boiling acetic anhydride and pyridine, but the amount was too small for purification.

The authors thank the Medical Research Council for the award (to R. M. A.) of a personal grant.

THE UNIVERSITY, NOTTINGHAM. [*Received, October 3rd,* 1949.]