the latter four peaks are due to olefins, formed by elimination[2] of water from four primary alcohols of molecular weights 326 ($C_{22}H_{46}O$, docosyl alcohol), 356 ($C_{24}H_{50}O$, carnaubyl alcohol), 382 ($C_{26}H_{54}O$, ceryl alcohol) and 420 ($C_{28}H_{58}O$, 1-octocosanol) respectively. The isolation of ceryl alcohol from the flowers of *C. fistula*[3] and myricyl alcohol ($C_{30}H_{50}O$) from *C. tora*[4] has also been reported.

Later fractions afforded a second white solid, m.p. 138–139°; $[\alpha]_D^{20}$ −46·5°; IR bands (KBr) ν_{max} 3450, 1645, 900, 810 cm^{-1}; NMR-($CDCl_3$) 4·7 τ, 5·52 τ, 9·00 τ, 9·05 τ, 9·42 τ, 9·23 τ, 9·32 τ. This material was identical with an authentic sample of β-sitosterol. (Found: C, 80·09; H, 11·02. Calc. for $C_{29}H_{50}O \cdot H_2O$; C, 80·19; H, −11·62%.) This was further confirmed by preparation of the acetate, m.p. 124–125°; $[\alpha]_D^{20}$ −40·6°; IR (KBr) ν_{max} 1730, 1650, 1250, 905 cm^{-1}; NMR ($CDCl_3$) 4·60 τ, 5·30 τ, 7·80 τ, 8·90 τ, 9·02 τ, 9·20 τ, 9·30 τ. (Found: C, 81·02; H, 11·1. Calc. for C_3, $H_{52}O_2$; C, 81·58; H, 11·40%.)

Leaves. The dried leaves were extracted after manner of Highet[5] and yielded a small amount of a brown oil which on TLC silica gel ($CHCl_3$–Et_2NH, 9:1) and on alumina ($CHCl_3$–MeOH, 9:1) showed two spots R_fs 0·72, 0·69 and R_fs 0·52, 0·47 in the two systems respectively. Authentic samples of cassine and dihydrocassine showed identical behaviour both when run alongside and when co-chromatographed. β-Sitosterol was also isolated, using the same extraction procedure as in the case of the flowers.

[2] R. A. FRIEDEL, J. L. SHULTZ and A. G. SHARKEY. *Anal. Chem.* **28,** 926 (1956)
[3] A. KUMAR, C. S. PANDL and R. K. KAND *Indian J. Chem.* **4,** 460 (1966).
[4] M. S. SASTRY. *Current Sci. India* **34,** 481 (1965).
[5] R. J. HIGHET. *J. Org. Chem* **29,** 471 (1964).

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THE FLAVONOIDS OF *CASSIA JAVANICA* FLOWERS

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(*Received* 18 *September* 1970)

Abstract—From the ethanolic extract of the flowers of *Cassia javanica* Linn. four flavonoid glycosides have been isolated and characterised by normal methods. The compounds have been found to be, leucocyanidin-4′-*O*-methyl ether-3-*O*-β-D-galactopyranoside; dihydrorhamnetin-3-*O*-β-D-glucopyranoside; quercetin-3′,4′,7-trimethyl ether-3-*O*-α-L-rhamnopyranoside; kaempferol-3-rhamnoglucoside. Quercetin was also obtained.

INTRODUCTION

PLANTS of the *Cassia* genus (Leguminosae; subfamily, Caesalpionoidae) are known to be a rich source of polyphenol and anthraquinone derivatives.[1] *Cassia javanica* Linn. is extensively used as a medicinal substitute of *Cassia fistula.*[2] With a view to study the nature of the constituents the chemical examination of the flowers was undertaken.

[1] O. P. YADAVA, Doctoral Thesis, University of Allahabad (1969)
[2] K. R. KIRTIKAR and B D BASU, *Indian Medicinal Plants*, Vol. II, p. 877, Leader Press, India (1935).

RESULTS AND DISCUSSION

The flowers of *Cassia javanica* Linn. collected and identified locally, have been studied and the presence of five flavonoid compounds, a leucoanthocyanin, *A*, a flavanonol glycoside, *B*, and three flavonol derivatives *C*, *D* and *E*, have been established. The compounds *A*, *B* and *C* are new glycosides.

Leucoanthocyanin A

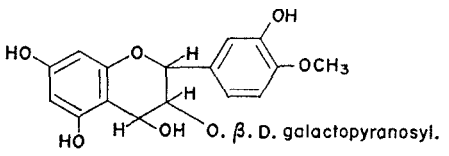
The fresh flowers of *Cassia javanica* were extracated with cold ethanol and the extract concentrated added to excess water. The aqueous solution was filtered from the coloured residue and fractionated by continuous liquid–liquid extraction. Light petroleum, 40–60° extracted some waxy material containing β-sitosterol. Subsequent extraction with ethyl acetate gave a product which on column chromatography on cellulose gave a good yield of *A*. Homogeneity of the compound was established by paper chromatography and TLC.

A gave a positive Molisch test and on hydrolysis with sulphuric acid it gave a sugar identified as D-(+)-galactose by paper chromatography and phenyl osazone derivative. Quantitative studies showed the compound was a monogalactoside in confirmity with the elemental analysis. *A* contained one methoxyl group (Ziesel; IR peak at 2850 cm^{-1}).[3] When treated with ethanolic hydrochloric acid it gave an anthocyanidin (I) which was demethylated with hydrobromic acid to second anthocyanidin (II) identified as cyanidin by colour reactions, paper chromatography and spectral studies.

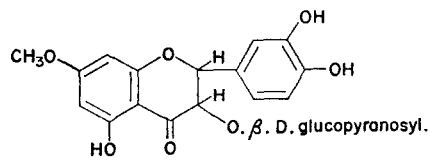
The bathochromic shifts with aluminium chloride showed the methoxyl group in *A* was in position-4'.[4] This was confirmed by isolation of isovanilic acid as one of the products on permanganate oxidation of *A*. The position of galactose was determined by polymerization behaviour, and alkali cleavage of *A*-methyl ether. Depending on the conditions of solvents and temperature, leucoanthocyanidins form polymers (75–90%) with mineral acids along with the corresponding anthocyanidins (10–25%).[5,6] Polymerization proceeds by the formation of an intermediate carbonium ion by the loss of OH at C_4. *A* formed a polymeric pigment on prolonged treatment with mineral acid, whereas, its completely methylated derivative did not. This showed that the hydroxyl at position-4 was not involved in the glycoside formation. The position of sugar moiety was established by methylation of *A* with diazomethane and alkali cleavage of the methylated product, when dimethyl phloroglucinol was obtained as one of the products showing free hydroxyl at position-5. As in many flavonoids[7] the hydroxyl at position-3 must be involved in the glycoside formation.

The glycoside did not give a positive colour reaction with aniline hydrogen phthalate showing the absence of a free aldehydic group in the sugar. The glycoside was hydrolysed with almond emulsin showing a β linkage to the phenol.[8] Quantitative oxidation studies of *A* with periodate showed the consumption of 2·1 moles of periodate with consequent liberation of 1·1 mole of formic acid for each mole of the glycoside. This suggested the pyranose form for galactose, also shown by peak at 822 cm^{-1} in the IR spectrum.[9]

[3] L. H. BRIGGS, L. D. COLEBROOK, H. M. FALES and W. C. WILDMAN, *Anal. Chem.* **29**, 904 (1954).
[4] T. A. GEISSMAN, E. C. JORGENSEN and J. B. HARBORNE, *Chem, & Ind.* 1389 (1953).
[5] D. G. ROUX and E. PAULUS, *Biochem. J.* **83**, 320 (1962).
[6] T. SWAIN and W. E. HILLIS, *J. Sci. Food Agri.* **10**, 63 (1959).
[7] J. B. HARBORNE, *Fortschr. Chem. Org. Naturst.* **20**, 165 (1962).
[8] S. M. HOPKINSON, *Quart. Rev.* **23**, 98 (1969).
[9] J. C. BURKET and R. M. BADGER, *J. Am. Chem. Soc.* **72**, 4397 (1950).



A



B

Flavanonol Glycoside B

The coloured residue obtained as described above was washed with water, dried and extracted with various solvents. Column chromatography of the concentrated acetone extract on magnesol gave the compound *B*. It was crystallized from chloroform and its homogeneity checked by paper chromatography and TLC. *B*, $C_{22}H_{24}O_{12}$ (λ_{max} 267,333 nm) on hydrolysis with ethanolic sulphuric acid gave an aglycone $C_{16}H_{14}O_7$ and D-(+)-glucose [paper chromatography and phenyl osazone]. The sugar:aglycone ratio was established as 1:1 by quantitative oxidation of *B* with periodate and also by the method of Pridham,[10] in agreement with the elemental analysis.

The colourless aglycone was shown to have a dihydro flavonol structure as indicated by the UV spectrum (λ_{max} 288,318 nm), borohydride and Pew reductions and other colour reactions. It was found to contain one methoxyl group (Ziesel) and formed a tetra acetate with acetic anhydride-pyridine reagents. The aglycone formed a tetramethyl ether with diazomethane, whereas, with dimethylsulphate a penta methyl ether was obtained. This indicates the presence of an alcoholic hydroxyl in the aglycone.

An examination of the spectra of the glycoside *B* and its aglycone revealed the presence of methoxyl group in position-7 (No shift with NaOAc),[11] a hydroxyl in position-5 (bathochromic shifts with $AlCl_3$)[11–13] and a 7-alkylated, 4'-OH skeleton.[11] The 3,5,7,3',4'-oxygenation pattern was indicated by alkali cleavage of the aglycone and neutral permanganate oxidation of its methyl ether, when monomethyl ether of phloroglucinol was found in the former and veratric acid in the latter case. This was confirmed by conversion of the aglycone into rhamnetin when treated with iodine and fused sodium acetate in glacial acetic acid by the method of Mahesh and Seshadri.[14]

The position of the glucose unit in *B* was determined by UV spectral shifts, and chemical reactions. The position of attachment of glucose moiety was shown to be the 3-OH group by comparing the effect of added $AlCl_3$ reagent on UV spectra of both *B* and its aglycone. With added $AlCl_3$, the dihydrorhamnetin spectrum changed considerably ($\Delta\lambda$ 52 nm) but the glycoside, lacking a 3-OH group showed a relatively smaller change ($\Delta\lambda$ 25 nm). The aglycone gave a positive Pacheco reaction due to free 3-OH group, whereas, the glycoside did not. This fact is also established by the behaviour of the glycoside towards alkali. Seshadri and coworkers[15,16] observed that 5-substituted or 5,7-disubstituted flavanones dissolve in 10% aq. sodium hydroxide on warming and are precipitated back in form of 2-hydroxy chalcones on acidification with dilute hydrochloric acid; however, the 5-hydroxy derivatives are recovered unchanged under these conditions.

[10] J. B. PRIDHAM, *Anal Chem.* **28**, 1967 (1956).
[11] R. M. HOROWITZ and L. JURD, *J Org Chem.* **26**, 2446 (1961).
[12] T. SWAIN, *Chem. & Ind* 1480 (1954).
[13] L. H. BRIGGS and T. P. CEBALO, *Tetrahedron*, **6**, 145 (1959).
[14] V. B. MAHESH and T. R. SESHADRI, *Proc. Indian Acad. Sci.*, **41A**, 210 (1955).
[15] N. NARASIMHACHARI and T. R SESHADRI, *Proc. Indian Acad. Sci.* **27**, 223 (1948); **30A**, 271 (1956).
[16] T. R. SESHADRI, *Sci. Proc Roy. Dublin Soc.* **27**, 77 (1956).

In the present study, the glycoside was recovered unchanged showing a free 5-hydroxyl and moreover, it has a 7-alkyl, 4′-OH skeleton.

The glycoside was completely hydrolysed with almond emulsin, showing β linkage between glucose and the dihydrorhamnetin. The quantitative oxidation of *B* with periodate showed the consumption of 2·0 moles of periodate with consequent production of 1·1 mole of formic acid for each mole of glycoside. This suggested a pyranose structure of the glucose moiety. The compound *B* represents one of the uncommon group of natural products, the 3-*O*-glycosylated dihydroflavonols.

Flavonol Glycoside C

The residue left after extraction of the compound *B* was exhaustively extracted with ethyl acetate. Excess of light petroleum was added to the concentrated extract giving a product which after repeated crystallizations from acetone–chloroform mixture gave compound *C*. Homogeneity of the compound was established by paper and thin layer chromatography.

Compound *C*, $C_{24}H_{26}O_{11}$, on acid hydrolysis, yielded a water insoluble aglycone $C_{18}H_{16}O_7$ and a reducing sugar which was identified as L-(−)-rhamnose by paper chromatography and phenyl osazone derivative. The yellow brown aglycone was assigned a flavonol structure by the study of its UV and visible spectra, reduction reaction and also by other reactions typical of flavonols. It was found to contain three methoxyl groups (Ziesel; IR peak at 2850 cm^{-1}) and two hydroxyls (diacetate with acetic anhydride-pyridine reagents).

An investigation of the spectra of the glycoside *C* (λ_{max} 268 and 357 nm) and its aglycone (255, 321 and 369 nm) indicated the presence of a methoxyl group in position-7 (No shift with NaOAc),[17] a free hydroxyl in position-5 (bathochromic shifts with $AlCl_3$),[17] and absence of free dihydroxyl system in positions-3′ and -4′ (lack of bathochromic shifts with boric acid-sodium acetate reagent).[18] The colour reactions of the aglycone are in conformity with the presence of two hydroxyls at positions-3 and -5 and one methoxyl in position-7. The other two methoxyl groups were fixed in positions-3′ and -4′ by permanganate oxidation of the aglycone, when veratric acid was isolated as one of the oxidation products.

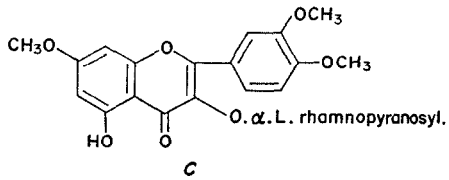
The position of rhamnose moiety in the glycoside was determined by spectral shifts[19,20] and complete methylation and hydrolysis of the rhamnoside. Complete methylation of the glycoside and subsequent acid hydrolysis of the methylated product, gave a tetra methyl ether agreeing in properties with the 5,7,3′,4′-tetra-*O*-methyl ether of quercetin.

The rhamnoside was not hydrolysed by emulsin showing the absence of β linkage. Hence, the probability is that there is α linkage between the aglycone and the rhamnose. This is in conformity with the fact that all the naturally occuring rhamnosides of known structure, so far reported, have α-configuration.[8,21,22]

Acid hydrolysis of the completely methylated derivative of the rhamnoside gave a methylated sugar which was identified as 2,3,4-tri-*O*-methyl-L-rhamnose. This suggested the rhamnose being linked through anomeric C_1 and having a pyranose structure which

[17] L. JURD and R. M. HOROWITZ, *J. Org. Chem.* **21,** 1395 (1956).
[18] L. JURD, *Arch. Biochem. Biophys.* **63,** 376 (1956).
[19] L. JURD and L. A. ROLLE, *J. Am. Chem. Soc.* **80,** 5227 (1958).
[20] L. JURD and R. M. HOROWITZ, *J. Org. Chem.* **22,** 1618 (1957).
[21] R. M. HOROWITZ and B. GENTILI, *Tetrahedron* **19,** 773 (1963).
[22] W. KLYNE, *Biochem. J.* **47,** 41 (1950).

was also indicated by quantitative oxidation of *C* with periodate. For each mole of rhamnoside, 2·1 moles of periodate were consumed and 1·2 moles of formic acid were produced.



C

Quercetin glycosides occur widely in nature and some glycosides of partial methyl ether of quercetin have also been reported.

Kaempferol-3-Rhamnoglucoside D

The aqueous solution left after extraction of the compound *A* was evaporated to a syrupy mass, which was extracted with acetone under reflux. The concentrated acetone extract on chromatography over silica gel gave a yellowish compound *D*. Acid hydrolysis of the compound *D* yielded a mixture of sugars consisting of D-(+)-glucose and L-(−)-rhamnose and an aglycone which was identified as kaempferol by its colour reactions, chromatography, IR, UV and visible spectra and preparation of derivatives.

Investigation of the spectra of *D* (λ_{max} 253, 315 and 355 nm) and its aglycone (267, 318 and 369 nm) indicated the presence of free hydroxyls in -5 and -7 positions. Spectrum of *D* was stable in sodium ethylate, and the visible region absorption band of the glycoside showed a shift of 14 nm when compared with the corresponding band of the kaempferol. Siegelman[23] has noted such shifts in spectra of flavonol and their 3-glycosides. The sugars were shown to be present as rutinose by hydrolysis of *D* with formic acid in cyclohexanol by the method of Hörhammer *et al.*[24] Quantitative oxidation of *D* with periodate and its almond emulsin hydrolysis in kaempferol indicated the pyranose structure of the sugars and β-linkage between kaempferol and the rutinose.

Kaempferol and its glycosides are well known compounds and they occur widely in *Cassia* genus.

Quercetin E

The residue left after extraction of the compound *C* was extracted with ether. The concentrated ether extract on standing deposited a green yellow substance, which on repeated crystallization from acetone–methanol mixture gave a good yield of *E*. Analysis of the compound showed it to be quercetin.

EXPERIMENTAL

Plant material. The fresh flowers of *Cassia javanica* were collected in full blossoms from the University campus in the months of April and May.

Chromatography. R_f values are related with ascending type of paper chromatography except in case of sugars, the solvents being: BuOH–HOAc–H_2O (4:1·5); Forestal; HOAc–conc. HCl–H_2O (30:3:10), HOAc–HCl–H_2O (5:1:5); 50% acetic acid and *n*-BuOH–EtOH–H_2O (5:1:4) Whatman No. 1 chromatostrips were used TLC was done using plates of Silica gel G.

Spectra IR spectra were recorded in KBr pellets UV and visible spectra were recorded using a Beckman mode DU spectrophotometer and absolute EtOH as the solvent. 1% EtOH–HCl and 1% MeOH–HCl,

[23] H. W. SIEGELMAN, *J. Biol. Chem.* **213,** 647 (1955).

[24] L. HORHAMMER, H. WAGNER and H. S. DHINGRA, *Arch. Pharm.*, **292,** 83 (1959).

were used in case of anthocyanidins. Spectral shifts in presence of various reagents were measured by the method of Jurd and Horowitz.[11,20]

Extraction of the flowers. The fresh flowers (1·2 kg) were directly extracted with EtOH at room temp. (32°) (10 × 24 hr) and the combined extract (3 × 5·0 l.) was concentrated under reduced pressure. The concentrated extract (600 ml) was poured in excess H_2O (1 l.) with continuous stirring as the result of which an aqueous solution (Fraction I) and a coloured residue (Fraction II) were obtained.

Fraction I. The water solution was continuously extracted with light petroleum, 40–60° till a colourless extract was obtained. On concentration, the solution yielded a product which revealed two spots in TLC (light petroleum–benzene 1:1, spray–2 N H_2SO_4). The crude product was chromatographed over neutral alumina and elution of the column with light petroleum–benzene (1:1) mixture gave a white compound, m.p. 134°. (Found: C, 83·75; H, 12·10. Calc. for $C_{29}H_{50}O$: C, 84·06; H, 12·08%.) It gave colour reactions of steroids and was identified as β-sitosterol (acetate, m.p. 127°; digitonide, m.p. 220°). This was confirmed by co-chromatography with authentic sample.

Ethyl acetate extract (leucocyanin). The aqueous extract from above was extracted with EtOAc. The concentrated extract was subjected to column chromatography on cellulose. Elution with EtOAc saturated with H_2O gave a product which was crystallized from EtOAc–light petroleum mixture as creamish white amorphous solid, m.p. 240° (dec.). The compound gave a positive Molisch test.

Both paper chromatography (R_f 0·51, BAW) and TLC (silica gel–5% aqueous sodium acetate, benzene–ethyl formate 75:25) revealed the presence of only one leucocyanin spot (vanillin in HCl). The compound gave positive reactions with formaldehyde–HCl and p-toluene sulphonic acid reagents and it had one methoxyl group. (Found: C, 54·98; H, 5·54; OCH_3, 6·1. Calc. for $C_{22}H_{26}O_{12}$: C, 54·77; H, 5·4; OCH_3, 6·3%). Leucocyanin had λ_{max} at 277·5 nm. IR spectrum showed the following main peaks : 3450 cm^{-1} (OH group), 2850 cm^{-1} (OCH_3 group), 1650 cm^{-1}, 1535 cm^{-1} and 1450 cm^{-1} (aromatic ring), 1130–1050 cm^{-1} (W), and 850–820 cm^{-1} (sugar moiety).

The leucocyanin (0·02 g) was refluxed with 10% ethanolic HCl (10 ml) for 2 hr. The anthocyanidin obtained (I) was purified by extracting in iso amyl alcohol and expulsion into 1% HCl with light petroleum. It had λ_{max} at 530 nm (MeOH–HCl) and at 540 nm (EtOH–HCl) and had R_f 0·56 (Forestal).

Anthocyanidin (I) was demethylated using 48%–HBr HOAc reagent in MeOH by refluxing for 1 hr. The product (II) gave all the colour tests of cyanidin; had R_fs 0·5 and 0·34 (Forestal and HOAc–HCl–H_2O); λ_{max} 536 nm (MeOH–HCl), 544 nm (EtOH–HCl) and showed bathochromic shifts with 1% $AlCl_3$ reagent, similar to authentic cyanidin. The identity was finally confirmed by co-chromatography with authentic cyanidin isolated from fresh rose-petal hydrolysate.

Leucocyanin (0·05 g) was acetylated with Ac_2O (5 ml) and pyridine (3 ml) at room temp. for 48 hr. The acetate crystallized from ethyl acetate–light petroleum mixture as colourless amorphous solid, m.p. 205° (dec.). (Found : C, 58·81; H, 5·1. Calc. for $C_{38}H_{42}O_{20}$: C, 55·74; H, 5·11%.)

Leucocyanin (0·2 g) in dry acetone (20 ml) was treated with CH_2N_2 and kept at 4° overnight, when a creamish product separated which was crystallized from acetone, m.p. 210° (dec.). (Found : OCH_3, 23·45. Calc. for $C_{21}H_{20}O_8$ $(OCH_3)_4$: OCH_3, 23·66%.) On refluxing (5% EtOH–HCl) a pink colour developed after 1 hr. The anthocyanidin was identified as tetramethyl cyanidin by paper chromatography and λ_{max} 533 nm (EtOH–HCl).

Complete methylation of the leucocyanin (0·05 g) with Me_2SO_4 and K_2CO_3 gave a methyl ether crystallized from acetone–MeOH mixture m.p. 185° (dec.). (Found : C, 60·54, H, 6·98. Calc. for $C_{30}H_{42}O_{12}$: C, 60·6; H, 7·07%.)

The leucocyanin on hydrolysis with 7% H_2SO_4 in EtOH for 2 hr at 100° gave D-(+)-galactose (R_f 0·16 BAW), which was confirmed by formation of phenyl osazone, m.p. 182° (lit. 186°) and co-chromatography with authentic sample.

The leucocyanin (0·05 g) dissolved in dry acetone (5 ml) was oxidized with 10% $KMnO_4$ under reflux for 4 hr. On working up isovanilic acid m.p. 252° (lit. 255°) was obtained. Confirmed by paper chromatography.[25]

2 N KOH under reflux for 2 hr gave phloroglucinol or its dimethyl derivative identified by chromatography.[26]

Leucocyanin in EtOH–H_2O (1:1) was treated with 0·1 M $NaIO_4$. The reaction mixture was allowed to stand at room temp. for 48 hr. The periodate consumed was determined by titration, and the formic acid liberated by the method of Hirst and Jones.[27]

Leucocyanin in EtOH was treated with crude emulsin solution (50 ml) prepared from sweet almonds[28] at 45–50° for 2 hr and then at room temp. for 4 days. The coloured product was removed with iso-amyl alcohol and the solution left gave a positive Molisch test shown due to D-(+)-galactose.

Acetone extract (kaempferol, 3-rhamnoglucoside). The aqueous extract after EtOAc extraction was evaporated to a syrupy mass and extracted with acetone under reflux. The concentrated extract was

[25] B. GENTILI, and R. M. HOROWITZ, *Tetrahedron*, **20**, 2313 (1964).
[26] N. A BURGES, H. M. HURST and B. WALDKEM, *Geochim. Cosmochim. Acta*, **28**, 1547 (1964).
[27] E. L. HIRST and J. K. N. Jones, *J. Chem. Soc.* **1659** (1949).
[28] F. G. MANN and B. C. SAUNDERS, *Practical Organic Chemistry*, p. 365, Longmann (1936).

chromatographed on silica gel. Elution with acetone–$CHCl_3$ (1:1) mixture gave a yellow product, crystallized from acetone–MeOH, m.p. 160°. It gave a deep red colour on treatment with Mg–HCl. It had R_f 0·54 (BAW, spray: ammonia). (Found: C, 54 62; H, 4·98. Calc. for $C_{27}H_{30}O_5$: C, 54·54; H, 5·05%.)

On hydrolysis it gave an aglycone (m.p. 280°) and L-(−)-rhamnose and D-(+)-glucose with 10% H_2SO_4 in EtOH.

Only rhamnose was obtained when hydrolysis was with formic acid in cyclohexanol.[24]

The enzymic hydrolysis of the glycoside with emulsin in the usual manner yielded the aglycone and rutinose [6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranose].

Aglycone. The crystalline aglycone gave different shades of red colour with Mg–HCl, Zn–HCl and Na–Hg–HCl. UV absorption curve and IR spectrum was identical with that of kaempferol The main IR peaks are, 3400 cm^{-1} (OH group), 1665 cm^{-1}, 1613 cm^{-1} (polyhydroxyflavone), 1560 cm^{-1}, 1515 cm^{-1} and 1450 cm^{-1} (aromatic ring), 1375 cm^{-1} and 820 cm^{-1}. (Found: C, 62·71; H, 3·52. Calc. for $C_{15}H_{10}O_6$: C, 62 93, H, 3·49%)

It formed kaempferol tetra acetate, m.p. 118° with Ac_2O and pyridine. (Found: $COCH_3$, 37·42. Calc. for $C_{15}H_6O_6$ $(COCH_3)_4$: $COCH_3$, 37·88%.) Methylation with Me_2SO_4 and K_2CO_3 gave tetramethyl ether of kaempferol, m.p. 210°. (Found: OCH_3, 35·98. Calc. for $C_{15}H_6O_2$ $(OCH_3)_4$: OCH_3, 36·25%.)

The alkali treatment of the kaempferol with 2 N KOH gave phloroglucinol and *p*-hydroxy benzoic acid.[29] Anisic acid was obtained on permanganate oxidation of the kaempferol methyl ether.

Fraction II. The coloured residue was separated from the water solution, washed with H_2O and subsequently dried. The dried residue was subjected to extraction with different organic solvents. Light petroleum removed waxy matter.

Acetone extract (flavanonol glycoside). The residual solid from above was continuously extracted with acetone and the solvent concentrated adsorbed on magnesol and eluted with acetone, MeOH and $CHCl_3$ respectively. The $CHCl_3$ eluate was found to contain a single entity as shown by TLC (benzene–ethyl acetate, 75:25), and on concentration, separated a colourless substance which was repeatedly crystallized from acetone–MeOH mixture as white amorphous solid, m.p. 170°. It had R_f 0·49 (BAW) and gave positive Shinoda, Pew and borohydride reductions. (Found: C, 55·2; H, 5 4. Calc. for $C_{22}H_{24}O_{12}$: C, 55; H, 5·0%.)

The compound gave a +ve Molisch test.

Acid hydrolysis. On hydrolysis it gave a colourless crystalline product, m.p. 255° and D-(+)-glucose (phenylosazone, m.p. 202°, lit. 204°). The sugar was quantitatively determined by the method of Pridham.[10]

Aglycone. The colourless crystalline aglycone gave positive Pacheco reaction, reduced Tollen's reagent and Fehling solution and gave other characteristic colour reactions of flavanonols. It had one methoxyl group. (Found: C, 61·1; H, 5·0; OCH_3, 8 1. Calc. for $C_{16}H_{14}O_7$: C, 60·3, H, 4 4; OCH_3, 9 74%.) Acetylation of the aglycone in the usual manner, gave a tetra acetate, m.p. 130°. (Found: $COCH_3$, 35·3. Calc. for $C_{16}H_{10}O_6$ $(COCH_3)_4$: $COCH_3$, 36 5%) Methylation of the aglycone with CH_2N_2 gave a tetramethyl ether, m.p. 156°. (Found: OCH_3, 34·7. Calc. for $C_{15}H_8O_3$ $(OCH_3)_4$: OCH_3, 35%.) Me_2SO_4–K_2CO_3 yielded a pentamethyl ether, m.p. 143°. (Found: C, 64·2: H, 5·64; OCH_3, 40·85. Calc. for $C_{20}H_{22}O_7$: C, 64 11, H, 5·8; OCH_3, 41·44%)

Alkali cleavage. The aglycone with 2 N KOH for 4 hr gave monomethyl phloroglucinol (lit., m.p. 78°) by mixed m.p. determination.

Permanganate oxidation The aglycone methyl ether (0 05 g) on oxidation with $KMnO_4$ gave veratric acid, m.p. 180° (lit., m.p. 182°).

Conversion into related flavone. The aglycone was dehydrogenated with I_2 and fused KOAc by the method of Mahesh and Seshadri.[15] The resulting product was crystallized from EtOH, m.p. 292°. It had λ_{max} at 258 and 372 nm, and showed positive bathochromic shifts with aluminium chloride (32 nm) and boric acid–sodium acetate (21 nm) reagents. Absorption curve and m.p. was identical with that of rhamnetin (lit., m p.: 295°). This was also in agreement with the colour reactions and chromatography behaviour (R_f 0·75 BAW) and its acetate, m.p. 202°. (Found: C, 60·6, H, 6·2. Calc. for $C_{16}H_{12}O_7$: C, 60·78, H, 6·15%.)

Ethyl acetate extract (rhamnoside of quercetin methyl ether). The acetone extracted residue was exhaustively extracted with EtOAc and the solution concentrated under reduced pressure and light petroleum added giving a brownish yellow solid from which EtOAc–light petroleum had m.p 190° (dec). Paper chromatography and TLC (benzene–ethyl acetate 75·25) revealed that the compound was homogeneous It had R_f 0·40 (BAW) and responded to colour reactions of flavones. It gave a positive Molisch test (Found: C, 58·68; H, 5·31. Calc. for $C_{24}H_{26}O_{11}$: C, 58·8; H, 5·3%.)

IR spectrum showed the following main peaks: 3350 cm^{-1} (OH group), 2850 cm^{-1} (OCH_3 group), 1604 cm^{-1}, 1500 cm^{-1} and 1450 cm^{-1} (aromatic ring), 1185 cm^{-1}, 1150 cm^{-1}–1130 cm^{-1} (W) and 835 cm^{-1}–810 cm^{-1}.

Hydrolysis. On hydrolysis it gave an aglycone, m.p. 205° and L-(−)-rhamnose.

Methylation and hydrolysis. Methylation (Me_2SO_4–K_2CO_3) gave a yellowish solid, m.p. 168–170°. (Found: C, 61·35; H, 6·1. Calc. for $C_{28}H_{34}O_{11}$: C, 61·53; H, 6·22%.) This methyl ether on hydrolysis gave an agly-

[29] H. M. HURST and J. B. HARBORNE, *Phytochem.* **6**, 1111 (1967).

cone, m.p. 192°, from acetone identified as 3,7,3′,4′-tetramethyl ether of quercetin by m.p. (lit. 192–194°) and spectral properties.[21,22] (Found C, 63·55; H, 5·2. Calc. for $C_{19}H_{18}O_7$: C, 63·68; H, 5·02%.)

2,3,4-tri-*O*-methyl-L-rhamnose was obtained as the sugar. On warming the solution with a mixture of aniline and aniline hydrochloride in methanol, a product was obtained which crystallized from light petroleum as colourless needles, m.p. 123° confirming the identity of the sugar (lit., m.p. 124–125°).[30]

Aglycone. The coloured aglycone gave characteristic colour reactions of 5-hydroxy flavonols with three methoxyl groups. (Found: C, 62·95; H, 4·5; OCH_3, 26·2. Calc. for $C_{18}H_{16}O_7$: C, 62·8, H, 4·65; OCH_3, 27·03%.) Acetylation with Ac_2O and pyridine gave a diacetate, m.p. 180°. (Found: $COCH_3$, 20·8. Calc. for $C_{18}H_{14}O_7$ $(COCH_3)_2$: $COCH_3$, 20·96%), and $KMnO_4$ gave veratric acid.

Ether extract (quercetin). The residue obtained from above was extracted with Et_2O yielding quercetin, m.p. 316°. (Found: C, 59·60; H, 3·31. Calc. for $C_{15}H_{10}O_7$: C, 59·46; H, 3·21%.) It formed a penta acetate, m.p. 195°. (Found: $COCH_3$, 39·88. Calc. for $C_{15}H_5O_7$ $(COCH_3)_5$: $COCH_3$, 41·21%.)

Acknowledgement—We are thankful to the Council of Scientific and Industrial Research (India) for the award of a research fellowship to one of us (O.P.Y.)

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SWARTZIAGENIN: A MIXTURE OF OLEANOLIC AND *O*-ACETYLOLEANOLIC ACIDS

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(*Received* 11 *January* 1971)

INTRODUCTION

THE SEED pods of the tree *Swartzia madagascariensis* Desv. have been reported to have been used effectively in controlling the populations of bilharzia-transmitting snails in African ponds.[1] Chemical studies by one of us have shown that the pods contain two acidic saponins, swartziasaponins A and B, which afford swartziagenin, a triterpene dicarboxylic acid of unknown constitution, on hydrolysis with Kiliani's reagent.[2,3] As part of a continuing study of saponins derived from tropical plants as potential molluscicides,[4] we have re-examined swartziagenin and have found it to be a mixture of oleanolic (Ia) and *O*-acetyloleanolic (Ib) acids.

[1] A. MOZELEY, *Molluscicides*, Lewis, London, p. 22 (1952).
[2] F. SANDBERG, B. AHLENIUS and R. THORSEN, *Svensk. Farm. Tidskr.* **62**, 541 (1958).
[3] F. SANDBERG, *Pakistan J. Sci. Indust. Res.* **4**, 258 (1961).
[4] *Report of the Tropical Products Institute* H.M.S.O., London, p. 13 (1967).