K_A and K_{mixed} are determined using the equations :

$$K_A = \frac{[VO(R)_2\,SCN]_{org}}{[VO(R)_2\,OH]_{org}\,[SCN^-]\,[H^+]},$$

(which is equivalent to)

$$\log \frac{E_A - E_{min}}{E_{max} - E_A} = \log K_A + \log [SCN^-] - pH$$

and

$$\log D_{mixed} = \log \frac{E}{E_{max} - E} = \log K_{mixed}$$

$$+ 2 \log [HR]_{org} + \log [SCN^-] - pH.$$

The value of log K_{mixed} is quite in agreement with the previous result.

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Leucoanthocyanins from *Cassia javanica* Root Bark

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FROM the ethanolic extract of the root bark of *Cassia javanica* two anthraquinones and their glycosides had been isolated and the chemistry of these compounds reported earlier[1].

In the present communication isolation and structural studies of two new leucoanthocyanins (I) and (II) are being reported.

Plant of the genus *Cassia* are well known for their medicinal value[2] and are also rich sources of flavonoids and anthraquinones[3,4,5].

The root bark of *Cassia javanica* was collected from a matured tree in the University campus. The dried powdered root bark was extracted with acetone, the extract concentrated under reduced pressure and poured into excess of cold water when a brown mass deposited which was filtered. The precipitate and filtrate were worked up separately.

The water insoluble portion was extracted with ether and ethyl acetate respectively. Ether fraction contained a flavonol, quercetin, which was confirmed by mp, mmp, derivatization and spectral studies[3]. Ethyl acetate fraction was concentrated and light petrol (40-60) was gradually added and the coloured sticky solid first separating was discarded. Further addition of petrol yielded the leucocyanin(I). The process was repeated several times in order to obtain an almost colourless sample, m. p. 290°(d.).

The water soluble portion was concentrated under reduced pressure and extracted with ethyl acetate in a continuous liquid-liquid extractor. Ethyl acetate extract was concentrated and petrol was gradually added and the coloured sticky solid first separated was discarded. Further addition of petrol yielded leucopeonin(II). The process was repeated several times till pure leucopeonin was obtained, melting at 200°(d.).

Leucocyanin (Compound I) :

Compound (I) $C_{21}H_{24}O_{11}$ has $[\alpha]_D^{30}$ – 120) (ethanol) ; R_f 0.46 (PC, BuOH : AcOH : H_2O :: 4:1:5° and 0.38 (TLC, Benzene : EtOAc :: 1:1) spray vanillin in HCl [6]. λ_{max}^{EtOH} 278 nm. IR ν_{max}^{KBr} 3450, 2900, 1650, 1540, 1130-1050, 850 and 820 cm^{-1}. Formed acetate with Ac_2O/pyridine m.p. 205° and methyl ether (Me_2SO_4/K_2CO_3) m.p. 180°.

Compound (I) on hydrolysis with 10% ethanolic hydrochloric acid gave an anthocyanidin and a free sugar rhamnose (PC, osazone). The anthocyanidin was identified as cyanidin by colour reactions, paper chromatography $\lambda_{max}^{EtOH-HCl}$ 515 nm and comparison with an authentic sample of cyanidin isolated from the red rose petals[7].

The position of the sugar moiety in the molecule was determined by subjecting the glycoside to catalytic hydrogenation (Pt/H_2)[8] under atmospheric pressure as well as under 15 lbs pressure when no free sugar could be detected. Therefore, the possibility of the sugar linkage at C_4 hydroxyl could be eliminated. The glycoside gave pink colour with *p*-toluene sulphonic acid reagent which showed that hydroxyl at C_5 is free[9]. This was also in conformity with the absorption that in the visible spectrum of the corresponding anthocyanidin a definite shoulder at 450 nm was obtained in addition to the main absorbance at 545 nm. On permethylation[10] and subsequent hydrolysis the glycoside yielded 5,7,3′,4′-tetra-*O*-methyl cyanidin[11] and 2,3,4-tri-*O*-methyl rhamnose indicating that the sugar is linked at C_3. The position of the sugar moiety was further confirmed by conversion of the glycoside into polymeric substance on prolonged acid treatment. The glycoside yielded a polymeric substance whereas the completely methylated glycoside lacked this property showing that sugar is not linked at C_4 of the diol unit[12]. It could thus be inferred that it is a 3-O-glycoside. This is also in agreement with the suggested biogenesis of these compounds[13] which favours position-3 for glycosidation. The methylated glycoside on alkali degradation yielded phloroglucinol dimethyl ether and veratric acid suggesting the attachment of rhamnose at C_3. That rhamnose is present in the pyranose form has been proved by periodate oxidation and nature of the glycosidic linkage as $\alpha-$ by hydrolysis with diastase. Compound(I) is thus leucocyanidin-3-O-α-L(−) rhamnopyranoside.

Compound (II) :

Compound(II) $C_{22}H_{26}O_{11}$ $[\alpha]_D^{28}-110$ (EtOH), R_f 0.54 (PC, BuOH, AcOH : H_2O :: 4:1:5) and 0.42 (tlc, benzene : EtOAc :: 1:1) (spray vanillin in HCl) λ_{max}^{EtOH} 275 nm υ_{max}^{KBr} 3440, 2910, 2880, 1650, 1530, 1450, 1180, 1130-1050, 850, and 825 cm^{-1}. It formed acetate (Ac_2O/Py) m.p. 180° and methyl ether (Me_2SO_4/K_2CO_3) m.p. 160°. On acid hydrolysis it gave rhamnose (PC, osazone) and an anthocyanidin which resembled natural peonidin in its colour reactions and $\lambda_{max}^{EtOH-HCl}$ 540 nm, co-chromatography with a natural sample of peonidin isolated from fresh viola tricolour flowers[14]. Alkali fission of the glycoside yielded phloroglucinol dimethyl ether and veratric acid suggesting the linking of rhamnose either at C_3 or C_4 positions of the leucopeonidin. This was decided in favour of position C_3 by catalytic hydrogenation and polymerisation of the glycoside with mineral acid. On periodate oxidation two moles of periodate were consumed per mole of the glycoside with liberation of one mole of formic acid which suggested the pyranose structure for rhamnose. The glycoside hydrolysed with diastase showing thereby the sugar linkage to be α. This is also in confirmity with the fact that all the naturally occurring rhamnosides of known structures so far reported have $\alpha-$ configuration[15]. Thus the compound (II) is leucopeonidin-3-O-α-L-(−) rhamnopyranoside.

These two glycoside (I) and (II) have not been reported earlier from any plant sources. The high astringent nature and medicinal uses of the root bark of *C. javanica* is most probably due to the occurrence of compound (I) (0.075%) and compound (II) (0·30%) in high yield.

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