second extraction (7.25 g.) was chromatographed on 800 g. of undeactivated Merck acid washed alumina, collecting fractions of 500 ml. Fractions 1–22 [benzene:chloroform (1:1)] and 23–25 [benzene:chloroform (1:2)] and 25–34 [benzene:chloroform (1:3)] gave 0.27 g., 0.03 g., and 0.34 g. respectively of intractable material.

Fractions 35–46 (chloroform) gave 1.06 g. of a crystalline brown solid. Recrystallized from ether 0.32 g. of *vallesin* was obtained in colorless needles, m.p. 153–156° not raised by further crystallization from hexane or by sublimation; $[\alpha]_D$ −92°, (*c*, 0.12 in chloroform); λ_{max} 259 mμ (log ε 4.11), infrared bands (chloroform) *inter al.* at 5.96 (vs), 6.02 (vs), 6.13 (s), 6.2 (vs), 6.66 (vs) and 6.76 (vs) μ.

Anal. Calcd. for $C_{21}H_{28}N_2O_2$: C, 74.08; H, 8.29; N, 8.23; O, 9.40; OMe, 9.11; (N)-Me, 4.42. Found: C, 73.97; H, 8.18; N, 8.21; O, 9.55; OMe, 8.72; (N)-Me, 1.49.

Fractions 47–51 [chloroform:methanol (97:3)] gave 2.14 g. of a brown semi-solid compound which upon trituration with ether gave 1.18 g. of a cream-colored crystalline compound. Recrystallized from hexane:benzene this material yielded aspidospermine, m.p. 206–208°, characterized by mixture melting point, infrared and ultraviolet spectra.

Fractions 51–62 [chloroform:methanol (95:5)], 63–67 [chloroform:methanol (9:1)], 68–75 [chloroform:methanol (4:1)], 76–82 [chloroform:methanol (7:3)] and 83–96 [chloroform:methanol (2:3)] provided respectively 0.67 g., 0.47 g., 0.21 g., 0.20 g., and 0.37 g. of brown amorphous solid from which no crystalline material could be obtained. It therefore seems likely that dichotamine present in the original mixture is too strongly adsorbed on undeactivated alumina to permit a satisfactory isolation and purification.

Stems. Using an extraction procedure exactly similar to that described for the leaves and twigs, 655 g. of ground stems gave 67 g. of total solid extract, which was processed as described above to give the following fractions: (1) A non-basic extract of hexane solubles (discarded). (2) Benzene-soluble acetates (1.6 g.). (3) Chloroform-soluble bases (2.2 g.). The paper chromatographic behavior of this fraction showed that it had a similar composition to the corresponding fraction from the leaves and twigs.

The benzene-soluble acetates were crystallized from 40 ml. of methanol giving 315 mg. of crude *reserpine*. After washing with hexane, the crude reserpine was recrystallized from methanol:benzene to give 115 mg. of still impure reserpine, m.p. 253–255° (dec.). The infrared spectrum and paper chromatogram were characteristic of authentic reserpine.

The benzene-soluble acetates were chromatographed on alumina but no crystalline fractions were obtained.

Stems taken from directly above the roots, (837 g.) were ground, extracted with methanol and processed as above to give the following fractions: (1) Non-basic hexane extract (5.7 g.) (not investigated). (2) Benzene-soluble acetates (0.80 g.). (3) Chloroform-soluble bases (5.5 g.). (4) Chloroform and water-insoluble bases (4.6 g.) (not investigated).

The benzene-soluble acetates showed no reserpine by paper chromatography.

The chloroform-soluble bases showed 6 components by paper chromatography but none of them appeared to be identical with those of the benzene-insoluble acetates from the previous stem extraction. Careful chromatography of this fraction on alumina, followed by counter-current partition of some of the chromatographic fractions gave only traces of crystalline material in amounts insufficient to characterize.

Roots (1220 g.) were ground, extracted with 2 × 6l. of hot methanol, and the extract (150 g.) was processed as previously described to give the following fractions: (1) Non-basic extract of hexane-solubles (9.2 g.) (not investigated). (2) Benzene-soluble acetates (1.05 g.). (3) Chloroform-soluble bases (8.55 g.).

The benzene-soluble acetates when chromatographed on paper showed 9 components but only traces of reserpine.

The chloroform-soluble bases showed 4 or more components when chromatographed on paper, but careful chromatography on alumina gave only intractable resins.

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[CONTRIBUTION FROM THE INDIAN ASSOCIATION FOR THE CULTIVATION OF SCIENCE]

Studies on the Ultraviolet Absorption Spectra of Coumarins and Chromones. II. Hydroxy Derivatives[1]

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Study of the ultraviolet absorption spectra of coumarins and chromones, substituted by hydroxy groups in the aromatic as well as in the heterocyclic nucleus in different positions shows bathochromic shift in the position of one or more of the principal bands.

In a previous communication[2] it has been shown that a methyl group substituting different positions in coumarin and chromone fails to cause any significant bathochromic shift of the main absorption bands. This was considered to be due to the weak auxochromic property of the methyl group.

The subject of the present investigation is a systematic survey of the absorption characteristics of coumarins and chromones having hydroxyl groups at different positions of the coumarin and chromone molecule.

The absorption spectra of some similar coumarins and chromones have been reported.[3–5]

The compounds studied have been listed in Tables I and II. Absorption was measured with a Beckmann Model DU quartz spectrophotometer

(1) Taken from the thesis submitted by Kalyanmay Sen for the degree of Doctor of Philosophy (Science) of the University of Calcutta, April 1957.

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using ethanol as the solvent at a concentration of about 5–6 mg. per liter in the region 220–360 mμ. Beyond this range no interesting features were, in general, observed. Preparation of the compounds following methods given in the literature is described under Experimental.

Results and discusssion. The absorption spectra of the hydroxycoumarins and hydroxychromones studied are given in Figures 1, 2, and 3.

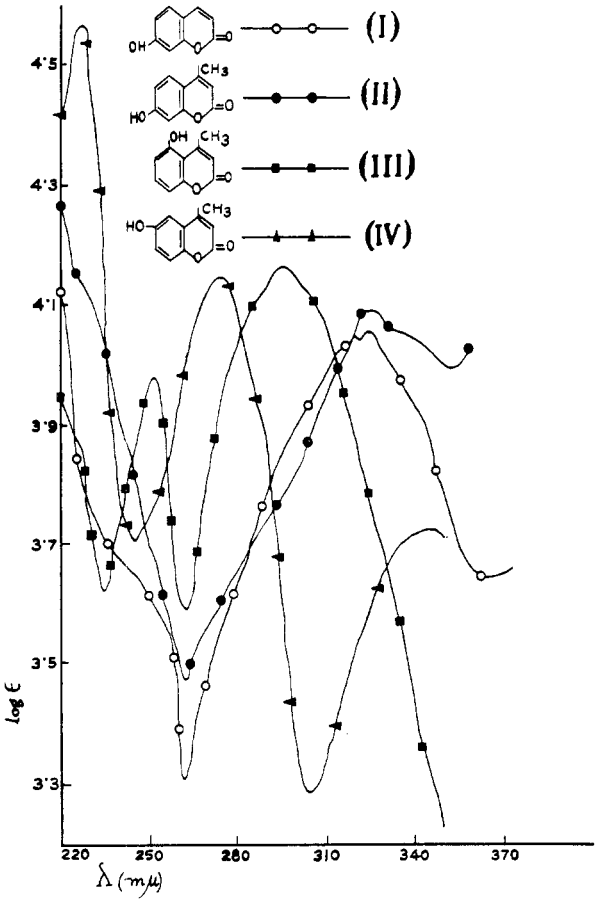

Fig. 1. Ultraviolet absorption spectra of I, 7-Hydroxycoumarin —○—○—; II, 7-Hydroxy-4-methylcoumarin —●—●—; III, 5-Hydroxy-4-methylcoumarin —■—■—■—; IV, 6-Hydroxy-4-methycoumarin —▲—▲—

It will be observed that the absorption spectrum of 6-hydroxy-4-methylcoumarin resembles those of coumarin and its methyl derivatives[2] quite closely. There is the characteristic minimum at 244 mμ(log ε = 3.71), the maximum at 275 mμ (log ε = 4.15), and the second minimum at 305 mμ. In addition there is to be found another very intense maximum at 227 mμ (log ε = 4.56) which is not observed in the spectra of coumarin and its methyl derivatives. This is perhaps the E band[6] shifted bathochromically due to the presence of the hydroxyl group in the aromatic nucleus. In the case of coumarin and its methyl derivatives this band presumably lies below 220 mμ. The band at

(6) E. A. Braude, *Ann. Repts.*, **42**, 105 (1945).

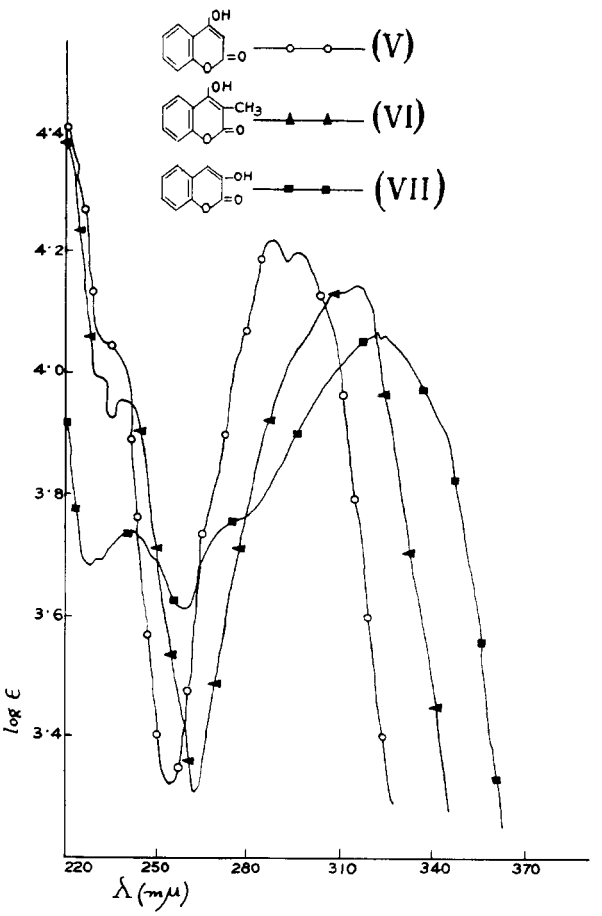

Fig. 2. Ultraviolet absorption spectra of V, 4-Hydroxycoumarin —○—○—; VI 4-Hydroxy-3-methylcoumarin —▲—▲—; VII, 3-Hydroxycoumarin —■—■—

275 mμ is presumably the K band and the low intensity one at 346 mμ the B band, which is shifted bathochromically compared to those in the spectra of coumarin and its methyl derivatives.

The spectra of 7-hydroxycoumarin and 7-hydroxy-4-methylcoumarin are very similar but are quite distinct from those of coumarin and its methyl derivatives. In both the first minimum lies around 261 mμ. In both the K and B bands have merged giving rise to one band. This contention is supported by the observation of twin peaks in the spectrum of 7-hydroxycoumarin. The presence of 4-methyl group in 7-hydroxy-4-methylcoumarin does not cause any significant change in the spectrum. A well-defined inflection at 240 mμ is observed in the spectrum of 7-hydroxycoumarin. The spectrum of the 4-methyl derivative shows a less distinct inflection at 228 mμ.

The spectrum of 5-hydroxy-4-methylcoumarin may be considered to fall in the class of the spectra of 7-hydroxycoumarin and its 4-methyl homolog. In this case the inflections observed in the spectra of 7-hydroxycoumarin and its 4-methyl homolog in the region 225–240 mμ has become resolved into a well defined band with maximum at 251 mμ (which we do not consider to be the E band because

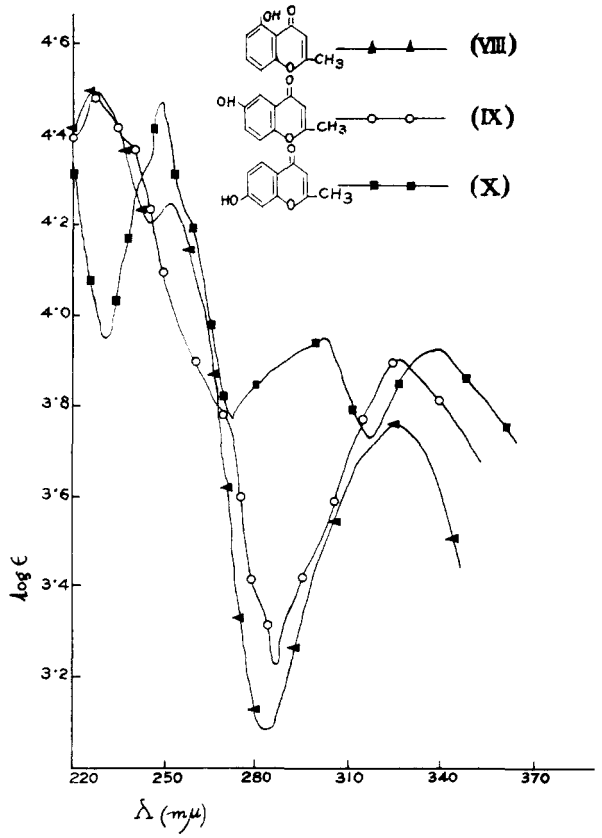

Fig. 3. Ultraviolet absorption spectra of VIII, 5-Hydroxy-2-methylchromone —▲—▲—; IX, 6-Hydroxy-2-methylchromone —○—○—; X, 7-Hydroxy-2-methylchromone —■—■—

of its low intensity). We believe that this band originates from some other structural feature of the molecule. The minimum at 262 mμ is observed. The second maximum occurs at a rather shorter wave length, λ_{max} 296 mμ, after which the absorption falls off.

The absorption spectra of hydroxycoumarins in which the hydroxyl groups occur in the heterocyclic nucleus *viz.*, 4-hydroxycoumarin, 4-hydroxy-3-methylcoumarin, and 3-hydroxycoumarin are plotted in Figure 2. It will be observed that the three curves are of similar nature, the characteristic minimum around 260 mμ is present in each case. At lower wave lengths the curves show the following features.

The curve for 4-hydroxycoumarins shows a distinct inflection at 231 mμ which develops into a wave with a short crest having λ_{max} at 293 mμ in the curve for 4-hydroxy-3-methylcoumarin. The resolution is still more pronounced in the case of 3-hydroxycoumarin where a well defined band with λ_{max} 241 mμ (log ϵ = 3.74) is observed. The other interesting fact about these curves is the occurrence of another intense band between the wave lengths 280 mμ and 325 mμ. These bands invariably show a twin crest and are presumably formed due to the merger of the K and B bands. On comparing the curves of 4-hydroxycoumarin and 4-hydroxy-3-methylcoumarin, it is found that this band is shifted bathochromically in case of the methyl derivative. The K-B band of 3-hydroxycoumarin is shifted still further, its λ_{max} occurring around 322 mμ. Beyond 325 mμ the absorption of all these three compounds rapidly falls off.

In Figure 3 are plotted the absorption curves of 5-hydroxy-, 6-hydroxy-, and 7-hydroxy-2-methylchromone. It will be observed that the curves for 5-hydroxy-2-methylchromone and 6-hydroxy-2-methylchromone are very similar in nature, the most pronounced feature being an intense maximum at 226 mμ (log ϵ = 4.49, E band) followed by a wave with a short crest in the case of the former with λ_{max} 252 mμ. There is a sharp minimum in both these cases between 284 and 286 mμ followed by a maximum around 326 mμ.

On comparing the spectra of the above compounds with those of chromone and its methyl derivatives, it will be observed that the curves are of similar nature both showing considerable amount of absorption in the region 220–250 mμ. The absorption then falls off giving rise to minima. The minima in the case of the two hydroxy compounds are shifted by about 10–15 mμ toward the higher wave length. The λ_{max} for the K-B bands for the hydroxychromones are also shifted bathochromically by about 20–30 mμ.

The absorption curve of 7-hydroxy-2-methylchromone would appear at first sight to be quite different from those of the other two hydroxychromones studied. This compound shows a sharp minimum at 230 mμ, a region in which the other two compounds show an intense maximum. It shows a sharp and intense maximum at 249 mμ (log ϵ = 4.47). It may be mentioned that 5-hydroxycoumarin also shows a λ_{max} at 252 mμ. It appears likely to us that the band at 249 mμ in the case of 7-hydroxy-2-methylchromone is the E band shifted bathochromically to a considerable extent. The band at 302 mμ in the case of 7-hydroxy-2-methylchromone very likely corresponds with the band at 252 mμ of 5-hydroxy-2-methylchromone. The third band of 7-hydroxy-2-methylchromone with λ_{max} at 340 mμ is evidently the K-B band shifted bathochromically by about 14 mμ.

Conclusions. The introduction of a hydroxyl group into the coumarin and the chromone molecule modifies the absorption characteristics causing in general bathochromic shift of the principal absorption bands.

Comparison of the spectra of 7-hydroxy- and 7-hydroxy-4-methylcoumarin, and of 4-hydroxy- and 4-hydroxy-3-methylcoumarin would reveal that the methyl group causes a bathochromic shift only in the case of the latter pair.

The absorption spectra of hydroxycoumarins differ from those of hydroxychromones in the following respects. The hydroxychromones show con-

siderable amount of absorption around 250 mμ, log ε being invariably greater than 4.1, while log ε in the case of hydroxycoumarins in this region is never greater than 3.75. The minima in the case of hydroxycoumarins have been found to lie between 255–262 mμ, whereas the minima in the case of chromone have been found to occur above 280 mμ.

Limaye and Kelkar[7] obtained both 5-hydroxy-4-methylcoumarin and 5-hydroxy-2-methylchromone from 2,6-dihydroxyacetophenone by the Kostanecki-Robinson reaction. The absorption curves of these compounds corroborate the structures assigned to these by the workers.

EXPERIMENTAL

All melting points are uncorrected. The compounds were repeatedly crystallized from the solvents noted under respective headings until sharp and constant melting points were obtained.

7-Hydroxycoumarin (I)[8] was crystallized from dilute ethanol; m.p. 226°; lit.,[8] 227–228°.

Anal. Calcd. for $C_9H_6O_3$: C, 66.66; H, 3.70. Found: C, 66.35; H, 3.79.

7-Hydroxy-4-methylcoumarin (II)[9] was crystallized from ethanol; m.p. 185°; lit.,[9] 185°.

Anal. Calcd. for $C_{10}H_8O_3$: C, 68.18; H, 4.55. Found: C, 68.13; H, 4.66.

6-Hydroxy-4-methylcoumarin (IV)[10] was sublimed under vacuum and then crystallized from glacial acetic acid; m.p. 245°; lit.,[10] 243°.

Anal. Calcd. for $C_{10}H_8O_3$: C, 68.18; H, 4.55. Found: C, 67.63; H, 4.57.

4-Hydroxycoumarin (V). To an ice cold suspension of ethyl sodioacetoacetate (prepared from 25 ml. ester, 5 g. sodium) in benzene (100 ml.) was added a benzene (50 ml.) solution of acetylsalicoyl chloride (prepared from 15 g. aspirin, 15 ml. thionyl chloride). The reaction mixture was refluxed over water bath for 7 hr. and then cooled and decomposed with ice cold hydrochloric acid (1:1). The organic layer was evaporated and the residual oil was boiled with 20% sulfuric acid (180 ml.) for 8 hr. The precipitated 4-hydroxy-3-acetylcoumarin was crystallized twice from ethanol m.p. 138°. (Yield 55%).

Anal. Calcd. for $C_{11}H_8O_4$: C, 64.72; H, 3.92. Found: C, 64.92; H, 3.93.

The above product (5 g.) was warmed with concentrated sulfuric acid (4 ml.) for 3–4 min. and the cooled solution was poured into excess water, whereby 4-hydroxycoumarin precipitated as a crystalline solid. It was crystallized twice from water; m.p. 210°; lit.,[11] 206°. (Yield 95%).

Anal. Calcd. for $C_9H_6O_3$: C, 66.66; H, 3.70. Found: C, 66.78; H, 3.5.

4-Hydroxy-3-methylcoumarin (VI)[12] was crystallized from dilute acetic acid; m.p. 231°; lit.,[12] 230°.

Anal. Calcd. for $C_{10}H_8O_3$: C, 68.18; H, 4.55. Found: C, 68.16; H, 4.46.

3-Hydroxycoumarin (VII). 3-Acetamidocoumarin prepared according to the method of Shaw *et al.*[13] was refluxed with 3*N* hydrochloric acid under nitrogen for 2 hr. The solution was kept at 5° overnight and the product filtered, washed with water, and crystallized from water; m.p. 153–154°; lit.,[14] 153°.

Anal. Calcd. for $C_9H_6O_3$: C, 66.66; H, 3.70. Found: C, 66.33; H, 3.93.

5-Hydroxy-4-methylcoumarin (III) *and 5-hydroxy-2-methylchromone* (VIII). These were obtained by slight modification of the method described by Limaye and Kelkar.[7] The Kostanecki product (1.5 g.) obtained from 2,6-dihydroxyacetophenone (2 g.), sodium acetate (2 g.), and acetic anhydride (4 ml.) after one crystallization from dilute acetic acid was refluxed with a solution of sodium carbonate (0.81 g. in 17 ml. water) for 1 hr. The insoluble matter m.p. 85° was filtered, sublimed in vacuum, and the sublimate crystallized from petroleum ether (40–60°) giving 5-hydroxy-2-methylchromone (VIII) as pale yellow needles; m.p. 92°.

Anal. Calcd. for $C_{10}H_8O_3$: C, 68.18; H, 4.55. Found: C, 67.96; H, 4.49.

The filtrate after removal of the above chromone was acidified to obtain a solid which after vacuum-sublimation and two crystallizations from ethanol, produced 5-hydroxy-4-methylcoumarin (III); m.p. 262°; lit.,[7] 262°.

Anal. Calcd. for $C_{10}H_8O_3$: C, 68.18; H, 4.55. Found: C, 67.98; H, 4.48.

6-Hydroxy-2-methylchromone (IX) was prepared following the details given by Desai and Mavani[15] employing hydroquinone diacetate prepared according to Amin and Shah.[16] It was crystallized from glacial acetic acid; m.p. 247°; lit.,[15] 247°.

Anal. Calcd. for $C_{10}H_8O_3$: C, 68.18; H, 4.55. Found: C, 67.77; H, 4.54.

7-Hydroxy-2-methylchromone (X) was prepared according to method of Tahara.[17] It was crystallized from pyridine after preliminary purification by vacuum sublimation; m.p. 252°; lit.,[17] 250°.

Anal. Calcd. for $C_{10}H_8O_3$: C, 68.18; H, 4.55. Found; C, 68.12; H, 4.62.

CALCUTTA 32, INDIA

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