ORIENTIN AND ISO-ORIENTIN FROM THE SEEDS OF *CROTALARIA LABURNIFOLIA* LINN.

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IN an earlier communication[1] from our laboratories, the isolation of orientin (lutexin), 8-C-glycosyl luteolin from the dehusked powder of the seeds of *Crotalaria laburnifolia* (Leguminosæ) was reported for the first time, and in continuation of that work we wish to record the results of our chemical investigation of the whole seeds in detail for other flavonoids present, since the husk portion of the seeds answered positive tests for this type of pigments. Further, the isolation of iso-vitexin from the fresh seeds of *C. anagyroides* has been recently reported[2] by us.

Dry seeds of *C. laburnifolia* (coarsely powdered in a Wiley cutter-mill in preference to disc grinders to avoid heating during comminution) were extracted four times with hot ethanol (80%) under reflux, and the total extract concentrated *in vacuo* till all the alcohol was removed. The aqueous concentrate was repeatedly shaken with light petroleum, ether, ethyl acetate and methyl ethyl ketone in succession. The fatty matter and carotenoid pigments were removed by light petroleum and ether, and the subsequent ethyl acetate and methyl ethyl ketone layers contained the flavonoid glycosides. The residue from the ethyl acetate extract was dissolved in hot ethanol and allowed to remain in an ice-chest for two days, when yellow crystalline solid, m.p. 255–57° was obtained (yield: 0·02%). This was identified as orientin by its colour reactions, behaviour on paper chromatography in different solvent systems, resistance to usual acid hydrolysis and direct comparison with an authentic sample of the compound earlier isolated.[1] The mother liquor was diluted with a little water and left in an ice-chest. After about two months, some yellow solid separated. This was twice recrystallised from aqueous alcohol, when dull yellow crystals, m.p. 235–37°, were obtained. It gave all colour reactions for a flavonoid glycoside and on hydrolysis with aqueous alcoholic sulphuric acid (7%), it gave quercetin (identified by direct comparison with an authentic sample and its acetate) and galactose (identified by paper chromatography). The glycoside as such could not be fully characterised for want of sufficient quantity of the pigment.

The residue from the methyl ethyl ketone extract was taken up in hot methanol and left in the ice-chest for about a week, when yellow crystalline solid was obtained. This was recrystallised twice from hot methanol, when yellow needles melting at 240–42° were obtained (yield, 0·1%). This could not be hydrolysed with 7% sulphuric acid in aqueous alcoholic medium for eight hours as well as with 25% hydrochloric acid. On paper chromatography in different solvent systems, it gave R_f values (Table I) agreeing with those of an authentic sample of iso-orientin,[3] and the colour reactions of the compound now isolated and iso-orientin were the same. The identity of the pigment as iso-orientin 6-c-glucosyl luteolin was further confirmed by IR and NMR spectra of our sample and an authentic sample of iso-orientin (homoorientin), at the Institute of Pharmaceutical Sciences of the University of Munich, and hydrolysis with hydriodic acid in phenol to give luteolin.

TABLE I

Chromatography of the pigment from C. laburnifolia *and authentic iso-orientin*

(Whatman No. 1 paper. ascending, temp. 30° ± 2°)

Solvent system	R_f values	
	Pigment from *C. laburnifolia*	iso-orientin (authentic)
15% acetic acid ..	·36	·36
60% " ..	·65	·65
*BAW 4 : 1 : 2 ..	·45	·45
*BAW 4 : 1 : 5 (upper) ..	·45	·46
*BAW 6 : 1 : 2 ..	·46	·46
Phenol saturated with water (lower)	·67	·67
†EFW ..	·66	·66
‡AHW ..	·74	·73
n Butanol : 27% acetic acid (1 : 1)	·49	·48

*BAW : *n*-Butanol : acetic acid : water.
†EFW : Ethyl acetate : formic acid : water (10 : 2 : 3).
‡AHW : Acetic acid : conc. hydrochloric acid : water (30 : 3 : 10).

Besides these flavonoids and the major alkaloid (Crotalaburnine) reported earlier,[1] we have detected 3 minor alkaloids by means of paper chromatography and thin layer chromatography on silica gel (Table II).

TABLE II
Chromatography of the alkaloids of C. laburnifolia

Solvent system	R_f values*	
	Crotalaburnine (major)	Minor alkaloids
Paper, Whatman No. 1, ascending		
1. *n*-Butanol saturated 5% acetic acid (upper phase)	0·61	0·20, 0·32, 0·50
2. *n*-Butanol : ammonia : water (30 : 1 : 5)	0·80	0·37, 0·51, 0·69
TLC—silica gel G-2 mm.		
1. Chloroform : methanol : ammonia (85 : 14 : 1)	0·55	0·08, 0·31, 0·67

* Spraying reagent : Dragendroff's (Munier modification).

It is interesting to note that the seeds of C. *laburnifolia* contain the flavonoid C-glycosides, orientin and iso-orientin and an O-glycoside of quercetin, whose occurrence in *Crotalaria* genus is reported for the first time. It may be mentioned here that the co-occurrence of orientin and hyperoside (quercetin 3-galactoside) in *Sarothamnus scoparius* (Papilionatæ) has been earlier recorded by Hörhammer *et al.*[4]

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VIBRATIONAL SPECTRA OF *o*-, *m*- AND *p*-FLUORO- AND BROMOBENZALDEHYDES

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THE electronir absorption spectra of *o*-, *m* and *p*-fluoro- and bromobenzaldehydes in the visible and the near-ultraviolet regions have been reported by a number of earlier workers.[1–5] So far no work appears to have been carried out on the infrared and Raman spectra of the three isomeric bromobenzaldehydes. The vibrational spectral studies of the three isomeric fluorobenzaldehydes have been made by Chandra[6] and Srivastava *et al.*,[7] but none of them has presented the vibrational assignments for these compounds. We have, therefore, recorded the infrared absorption spectra of these six compounds in the region 400-4600 cm.$^{-1}$ and vibrational assignments have been made.

The infrared absorption spectra of the three isomeric fluorobenzaldehydes were recorded in the region 400–700 cm.$^{-1}$ on a Carl-Zeiss (Model UR 10) double beam infrared spectrophotometer using a thin film of pure liquid enclosed between two KBr windows, and the infrared spectra of *o*- and *m*-bromobenzaldehydes were recorded in the same region on a Perkin-Elmer infrared spectrophotometer (Model 21) with KBr prism using a 0·10 mm. cell. The infrared absorption spectra of the three isomeric fluorobenzaldehydes and *o*- and *m*-bromobenzaldehydes in the region 700–4600 cm.$^{-1}$ were recorded on a Perkin-Elmer spectrophotometer (Model 13 U) with NaCl prism using a 0·05 mm. cell. The infrared spectrum of *p*-bromobenzaldehyde was recorded in the region 700–4600 cm.$^{-1}$ on a Perkin-Elmer spectrophotometer (Model 13 U) with NaCl prism using KBr pellet technique. The accuracy of measurement is 2 cm.$^{-1}$ between 400–1500 cm.$^{-1}$, 4 cm.$^{-1}$ between 1500–3000 cm.$^{-1}$ and 10 cm.$^{-1}$ above 3000 cm.$^{-1}$.

Benzaldehyde[8] as also *p*-fluoro- and *p*-bromobenzaldehydes belong to C_{2v} point-group to a first approximation. The total number of 36 vibrations are divided into 13 a_1, 12 b_1, 4 a_2 and 7 b_2 classes, which are all allowed in the Raman spectrum and all but a_2 in infrared spectrum. On the reduction of symmetry the *o*- and *m*-fluoro- and *o*- and *m*-bromobenzaldehydes belong to C point-group and give 25 a' planar and 11 a'' non-planar vibrations.