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# C-GLYCOSIDES OF THE LEAVES OF *PARKINSONIA ACULEATA*

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**Abstract**—The leaves of *Parkinsonia aculeata* have been found to contain three C-glycosides. Two, could be separated by fractional crystallization after preliminary lead salt purification, but preparative paper chromatography was found effective for the separation of all three. The first (*epi-orientin*) is a C-glucoside of luteolin and resembles orientin in composition, chemical reactions and UV and IR spectra but differs in m.p. and rotation and may be an epimer of orientin. The second (*Parkinsonin-A*) is a C-glucoside of 5-O-methylluteolin and is closely related to orientin. The third (*Parkinsonin-B*) is a C-glucoside of 5,7-di-O-methylluteolin and has stereochemistry related to epi-orientin.

*Parkinsonia aculeata*, belonging to the Leguminosae, is a glabrous bush or low tree and a native of tropical America,[1] although now almost naturalized in India especially in the hotter regions. Hot ethanol extracts all the pigment from the fresh leaves. From the concentrate chlorophyll and waxy matter may be removed with light petroleum or better with ether itself since there is no other ether extractable matter. Circular paper chromatography of the remaining solution indicated the presence of three pigments with $R_f$ values 0·54, 0·70 and 0·94.

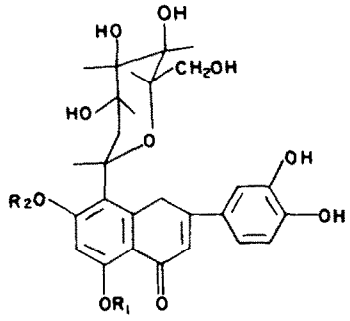
No solid crystallized out from the concentrate. Though all the pigments could be extracted with ethyl acetate, fractional crystallization did not yield pure products. From a dilute aqueous concentrate the pigments as lead salts were precipitated with neutral lead acetate leaving a small amount in the basic lead acetate precipitate. These lead salts were decomposed in ethanolic solution with hydrogen sulphide yielding a coloured semisolid. Further purification was effected by extracting an aqueous solution with ethyl acetate from which a crystalline yellow solid was obtained. By fractional crystallization from t-butanol, the first two pigments could be separated in a pure state, though in poor yield. A more effective method for the separation of all three pigments was paper chromatography.

The colour reactions of all the three components indicated that they were polyphenolic and belonged to the flavonoids. Positive Molisch's test and their stability towards acidic and enzymatic hydrolysis suggested that all the three were C-glycosides. Since the separation was difficult the mixture of the pigments was subjected to ferric chloride oxidation and glucose was found as the only product by chromatography and the formation of osazone. It could, therefore, be concluded that all the pigments contain glucose as the sugar moiety.

The first pigment, m.p. above 300°, has the molecular formula $C_{21}H_{20}O_{11}$ and is devoid of methoxyl groups. Its UV spectrum is very similar to that of luteolin. Methylation with diazomethane affords a tetramethyl ether which gives no ferric reaction but still shows the presence of hydroxyl groups in the IR spectrum. These are alcoholic hydroxyl groups as proved by the preparation of the acetate which is

[1] J. D. Hooker, *The Flora of British India* Vol. II; p. 260. L. Reave, Kent (1879).

hydroxyl free. Fission of the compound with hydriodic acid in phenol gives a quantitative yield of luteolin. The glycoside closely resembles orientin, 8-C-glucoside of luteolin in UV and IR spectra and paper chromatography. Further, by the periodate–borohydride degradation[2] glycerol is obtained. Glucose is the only sugar involved in the glycoside. The tetramethyl ether consumes 2·1 moles of sodium metaperiodate and yields 1·08 moles of formic acid proving conclusively that it is luteolin-8-C-glucopyranoside(I). Since it differs from orientin in m.p. and rotation, it is probably *epi-orientin*.




I $R_1, R_2 = H$

II $R_2 = H$; $R_1 = CH_3$

III $R_1, R_2 = CH_3$

The second pigment with $R_f$ 0·70 and m.p. 224° is a new compound and has been named *Parkinsonin-A*. It has the molecular formula $C_{22}H_{22}O_{11}$, is laevorotatory and contains one methoxyl group. It resembles luteolin-8-C-glucoside, orientin, very closely in the IR and UV spectra. Methylation of parkinsonin-A with diazomethane affords a trimethyl ether which agrees closely with orientin tetramethyl ether in UV and IR spectra and yields glycerol by treatment with periodate and borohydride. Fission of the pigment with phenol and hydriodic acid gives a good yield of luteolin. The position of the methoxyl group is considered to be 5 since its UV spectrum shows no bathochromic shift with aluminium chloride. This is further supported by the fact that complete methylation of the phenolic hydroxyl groups takes place more easily than with epi-orientin. When subjected to oxidation with sodium metaperiodate the trimethyl ether, 2·06 moles of periodate are consumed with the liberation of 0·98 mole of formic acid. Tetra-O-methyl orientin and *Parkinsonin*-A trimethyl ether had the same m.p. and mixed m.p. *Parkinsonin*-A is, therefore, 5-0-methyl luteolin-8-C-glucoside(II) with glucose in the pyranose form.

The third pigment, $R_f$ 0·94 and m.p. >300°, is also a new compound and has been named *Parkinsonin-B*. It has the molecular formula $C_{23}H_{24}O_{11}$ and has two methoxyl groups. It resembles epi-orientin in the IR and UV spectra but shows no bathochromic shift with sodium acetate or with aluminium chloride indicating an absence of free 5- and 7-hydroxyl groups. Its dimethyl ether obtained by the diazomethane method may be degraded in a stepwise manner to give glycerol. Since the amount of *Parkinsonin*-B was insufficient for hydriodic acid fission, the mixture of the three

[2] V. K. Bhatia, S. R. Gupta and T. R. Seshadri, *Curr. Sci.* 33, 581 (1964).

pigments was subjected to reductive cleavage. Isolation of only luteolin as the fission product led to the conclusion that *Parkinsonin*-B is also a derivative of luteolin, the positions of the two methoxyl groups being 5 and 7. The nature of the sugar ring as pyranose was revealed by the oxidation of the dimethyl ether with sodium meta-periodate when it consumed 2·1 moles of periodate with liberation of 1·08 moles of formic acid. Since epi-orientin tetramethyl ether and *Parkinsonin*-B dimethyl ether are identical in all respects, parkinsonin-B is 5,7-di-O-methyl epi-orientin(III).

Thus the leaves of *Parkinsonia aculeata* contain epi-orientin, 5-O-methyl orientin and 5,7-di-O-methyl epi-orientin. The most striking structural feature of the two methyl ethers is the methylation of the 5-hydroxyl group which is normally considered to be less reactive owing to chelation. It has been earlier suggested that in such cases where the 5-hydroxyl group suffers preferential methylation or glycosidation, these processes occur in biogenesis before the pyran ring closure.[3,4]

TABLE 1. COMPARISON OF PROPERTIES OF ORIENTIN AND THE THREE C-GLYCOSIDES FROM *P. aculeata* AND THEIR DERIVATIVES

| Compound | M.P. | Rotation |
|---|---|---|
| Orientin[5] | 265–267° | $[\alpha]_D^{20}$ +18·4 ± 0·9° |
| Orientin octaacetate[5] | 196–197° | $[\alpha]_D^{20}$ −54·4° |
| Orientin tetramethyl ether[5] | 273–276° | $[\alpha]_D^{20}$ +50·7° |
| Epi-orientin | >300° | negligible |
| Epi-orientin acetate | 191–192° | $[\alpha]_D^{22}$ −63·3° |
| Epi-orientin tetramethyl ether | 259° | $[\alpha]_D^{22}$ −39·7° |
| *Parkinsonin*-A | 224° | $[\alpha]_D^{22}$ −32° |
| *Parkinsonin*-A acetate | 144–145° | $[\alpha]_D^{22}$ +25·7° |
| *Parkinsonin*-A trimethyl ether | 273–274° | $[\alpha]_D^{22}$ +51·1° |
| *Parkinsonin*-B | >300° | negligible |
| *Parkinsonin*-B dimethyl ether | 258–259° | $[\alpha]_D^{28}$ −40·3° |

*Note on stereoisomerism in the C-glycosides*

The m.ps and rotations of orientin and the three compounds isolated are presented in the Table and the following results are indicated. Epi-orientin resembles orientin in composition, chemical reactions, UV and IR spectra but they differ in m.ps and rotations. A similar difference is noted in their tetramethyl ethers. Since methylation employed only diazomethane, the methyl ethers should have the same configuration as the original compounds. As the acetates resemble one another more closely, interconversion during acetylation may take place. It is quite possible that epi-orientin is an epimer of orientin.

*Parkinsonin*-A trimethylether agrees with orientin tetramethyl ether in composition, UV and IR spectra, m.p. and rotation, showing that *Parkinsonin*-A is related to orientin.

The dimethyl ether of *Parkinsonin*-B is identical in every respect with the tetramethyl ether of epi-orientin and they should, therefore, have the same stereochemistry. *Parkinsonin*-B is, therefore, epi-orientin-5,7-dimethyl ether.

[3] T. R. Seshadri, *Ann. Rev. Biochem.* **20**, 487 (1951).
[4] T. R. Seshadri, *Tetrahedron* **6**, 169 (1959).
[5] B. H. Koeppen, C. J. B. Smit and D. G. Roux, *Biochem. J.* **83**, 507 (1962).

## EXPERIMENTAL

The $R_f$ values were determined by circular paper chromatography on Whatman No. 1 filter paper. The solvent systems were water saturated with phenol (a), phenol saturated with water (b), n-butanol: acetic acid: water (4:1:5), butanol layer (c) and aqueous layer (d), 30% acetic acid (e), 15% acetic acid (f) and ethyl acetate: pyridine: water (10:4:3, v/v) (g). The flavonoid compounds were developed by exposure to ammonia vapour. The UV spectra taken in EtOH are marked (i), with added sodium acetate (ii), with added $AlCl_3$ (iii), with boric acid and sodium acetate (iv) and with EtONa (v); log $\varepsilon$ values are given in brackets. Light petroleum (40–60°) has been used.

*Extraction.* Fresh leaves (1·7 kg) were extracted with boiling alcohol (3 × 3 l., 8 hr each time). Chlorophyll and waxy matter were removed from the concentrate by washing with light petroleum (5 × 500 ml) and ether (10 × 500 ml). Chromatography of the aqueous solution in (a) gave three rings with $R_f$ values 0·54 (epi-orientin), 0·70 (*Parkinsonin*-A) and 0·94 (*Parkinsonin*-B). A solution of neutral lead acetate (100 g, 150 ml water) was added to the aqueous solution (450 ml). The dull yellow lead salt was macerated with EtOH to remove alcohol soluble impurities, filtered and decomposed in EtOH (600 ml) by passing $H_2S$ (1 hr). The process of decomposition was repeated 3 times more and alcohol was removed from the combined solutions under red. press. The dark brown semisolid residue was taken up in hot water (50 ml), the aqueous solution decanted off from insoluble dark brown non-flavonoid material and continuously extracted with ethyl acetate (1 l., 10 × 24 hr) changing the solvent after every 24 hr. The combined ethyl acetate extracts were concentrated to a small volume when the mixture of the pigments separated as a brownish yellow solid (3·4 g).

The neutral lead acetate filtrate was then treated with a solution of basic lead acetate (100 g, 2 l. water). The yellow lead salt on decomposition and purification as before gave a very small amount of the mixture of the pigments, 450 mg. The water insoluble resinous material was a significant part of this fraction.

*Isolation of epi-orientin and parkinsonin-A.* The mixture of the pigments (3·85 g) was taken up in t-butanol (70 ml). The solution was concentrated in stages and 3 major fractions collected: the brownish yellow first fraction contained all 3 pigments, the yellow second fraction predominated in epi-orientin and a yellow third fraction predominated in *Parkinsonin*-A. The second fraction on crystallization from abs. EtOH yielded pure epi-orientin (80 mg). The third fraction on crystallization from 95% EtOH gave pure parkinsonin-A (130 mg). The mixture of the pigments was recovered from the mother liquors by precipitation with ethyl acetate.

*Separation of the pigments by paper chromatography.* The mixture of pigments (1·2 g) was placed on Whatman No. 3MM strips (400) and descending chromatography performed in (a) at 25°. The strips were air dried, exposed to ammonia vapour and the zones (from top to bottom) corresponding to the pigments A, B and C cut. They were then separately extracted with light petroleum (2 × 1·5 l., 4 hr each time) to remove the phenol. After removing the light petroleum completely, the zones were extracted with MeOH (4 × 2 l., 6 hr each time). The combined MeOH extracts from each zone were concentrated to 5–6 ml when the pigment separated out; epi-orientin recrystallized from abs. EtOH as pale yellow globular clusters m.p., >300° (250 mg, 0·13% of air-dry leaves); *Parkinsonin*-A from 95% EtOH as yellow microcrystalline solid, m.p., 224° (dec) (500 mg, 0·26%) and *Parkinsonin*-B from MeOH as yellow prisms, m.p., >300° (180 mg., 0·09%).

### *Epi-orientin*

It was very soluble in $Na_2CO_3$aq and NaOHaq giving deep yellow solutions. With alcoholic $FeCl_3$ it gave a green colour, with Mg and HCl a reddish pink colour and it gave a positive Molisch's test. UV data: (i) 258, 269 (infl) and 352 m$\mu$ (4·1, 4·1 and 4·1); (ii) 279, 325 and 394 m$\mu$ (4·2, 3·9 and 3·8); (iii) 276 and 394 m$\mu$ (4·1 and 4·1); (iv) 264 and 374 m$\mu$ (4·2 and 3·9); (v) 275 and 407 m$\mu$ (4·2 and 4·1), IR (KBr disc): 3571 (OH); 1653 (conjugated CO); 1613, 1562, 1504 $cm^{-1}$ (aromatic bands). It did not show appreciable rotation. (Found: C, 56·8; H, 4·8. Calc. for $C_{21}H_{20}O_{11}$: C, 56·4; H, 4·5%.) The pigment and an authentic sample of orientin had the same chromatographic behaviour in solvent systems a, b, c, d and f having $R_f$ values 0·53, 0·67, 0·52, 0·57 and 0·41 (20°).

Epi-orientin (80 mg) was acetylated using acetic anhydride (2 ml) and dry pyridine (1 ml) at 100° for 4 hr. Repeated crystallization of the acetate from aqueous acetic acid gave colourless prismatic needles, m.p. 191–192° (68 mg). It gave no colour with alcoholic $FeCl_3$; $[\alpha]_D^{28}$ −63·3° (9·48 mg, 1 ml, pyridine) IR (nujol mull): 1752 (acetate C=O) 1650 (conjugated CO) and 1215 $cm^{-1}$ (acetate C=O). (Found: C, 56·8; H, 4·8. Calc. for $C_{27}H_{30}O_{19}$: C, 56·6; H, 4·6%.)

Epi-orientin (50 mg) in abs. MeOH (30 ml) was methylated using diazomethane (from 2·5 g of nitroso methyl urea) in ether (100 ml). At the end of 24 hr more diazomethane in ether was added till the solution was distinctly yellow. After 72 hr diazomethane and solvents were removed by evaporation. Recrystallization of the methyl ether from dry acetone gave colourless needles, m.p., 259° (28 mg). It gave no colour with alcoholic $FeCl_3$; $[\alpha]_D^{28}$ −39·7° (6·55 mg, 1 ml pyridine); UV (i) 246, 266 and 334 m$\mu$ (4·5, 4·4 and 4·4); IR (KBr disc): 3509 (OH); 1639 (conjugated CO); 1592, 1504, 1449 (aromatic bands); 1042 cm$^{-1}$ ($OCH_3$). (Found: C, 55·0; H, 5·9; $OCH_3$, 19·5. $C_{25}H_{28}O_{11}\cdot 2H_2O$ requires: C, 55·5; H, 5·9; $4OCH_3$, 22·9%.)

*Action of hydriodic acid.* To the suspension of epi-orientin (100 mg) in phenol (0·6 g), HI (1 ml, *d* 1·7) was added gradually with cooling. The mixture was gently refluxed (135–137°) for 7 hr and poured into $NaHSO_3$aq. Repeated crystallization of the product first from aqueous MeOH and then MeOH gave yellow needles, m.p., >300° (28 mg). With alcoholic $FeCl_3$ it gave an olive green colour and with Mg and HCl a pink colour. UV data: (i) 256, 268 (infl) and 352 m$\mu$ (4·2 and 4·1); (ii) 269 and 400 m$\mu$ (4·2 and 4·3); (iii) 275 and 390 m$\mu$ (4·3 and 4·3); (iv) 262 and 372 m$\mu$ (4·2 and 4·4); (v) 270 and 410 m$\mu$ (4·2 and 4·1); IR (nujol mull): 3571 (OH); 1660 (conjugated CO); 1613, 1562 and 1504 cm$^{-1}$ (aromatic bands). The aglycone and an authentic sample of luteolin had the same chromatographic behaviour in solvent systems a, b, c, d and e with $R_f$ values 0·42, 0·83, 0·29, 0·92 and 0·47. Acetylation of the aglycone using acetic anhydride and pyridine and recrystallization of the acetate from EtOH afforded colourless needles, m.p. and mixed m.p. with authentic luteolin tetra-acetate, 222–224°.

*Periodate oxidation of epi-orientin tetramethylether followed by borohydride reduction.* To a solution of the methyl ether (2 mg) in water (0·2 ml) was added sodium metaperiodate (2 mg) and the mixture kept at room temp for 4 hr. Sodium borohydride (2 mg) in water (0·1 ml) was added, the solution kept at room temp overnight and then heated with 1N HCl (0·2 ml) at 100° for 15 min. Ascending chromatography of this solution with (g) as solvent and as spray a mixture of aqueous sodium metaperiodate (2%, 4 parts) and alkaline $KMnO_4$ (1% $KMaO_4$ in 2% $Na_2CO_3$ aq, 1 part) gave a yellow spot on a pink background with $R_f$ 0·38 (30°). Glucose treated in the same manner gave $R_f$ 0·37 and so also its mixture with epi-orientin.

*Periodate oxidation and the estimation of formic acid.* Epi-orientin tetramethylether (4·1 mg) was dissolved in peroxide-free dioxan (1 ml) and water (9 ml), sodium metaperiodate (10 moles, 5 ml) added and the mixture kept at room temp (22°) for 24 hr. An aliquot (5 ml each time) was taken for titration using 0·063N sodium arsenite solution (10 ml) and 0·049N $I_2$ to calculate the number of moles of periodate consumed. In another experiment, methyl ether (7·97 mg) was dissolved in peroxide-free dioxan (1 ml) and water (9 ml), sodium metaperiodate solution (10 moles, 5 ml) added and the solution kept at 22° for 24 hr. After this interval, the solution was titrated potentiometrically against 0·011N KOH to calculate the number of moles of formic acid produced. The tetramethyl ether consumed 2·1 moles of periodate with the liberation of 1·06 moles of formic acid.

*Parkinsonin-A*

It was soluble in $Na_2CO_3$aq and NaOHaq giving deep yellow solutions. With alcoholic $FeCl_3$ it gave a green colour, with conc $H_2SO_4$ a yellow solution with green fluorescence, with Mg and HCl a pink colour and it gave a positive Molisch's test. $[\alpha]_D^{28}$ −32° (5·2 mg, 1 ml, pyridine); UV data: (i) 258, 271 and 352 m$\mu$ (4·5, 4·5 and 4·6); (ii) 278, 326 and 395 m$\mu$ (4·6, 4·3 and 4·5); (iii) 267, 279 and 360 m$\mu$ (4·6, 4·6 and 4·6); (iv) 265, and 376 m$\mu$ (4·5 and 4·5); (v) 270 and 412 m$\mu$ (4·1 and 4·2); IR (nujol mull): 3571 (OH); 1653 (conjugated CO); 1587, 1575 cm$^{-1}$ (aromatic bands). The pigment had $R_f$ values of 0·71, 0·78, 0·73, 0·68 and 0·56 in solvent systems a, b, c, d and f (20°). (Found: C, 54·6; H, 4·6. $C_{22}H_{22}O_{11}\cdot H_2O$ requires: C, 55·0; H, 5·0%.)

*Parkinsonin*-A (50 mg) formed an acetate which recrystallized from aqueous acetic acid as colourless needles, m.p., 144–145° (40 mg). It gave no colour with alcoholic $FeCl_3$. $[\alpha]_D^{28}$ +25·7° (7 mg, 1 ml pyridine); IR (nujol mull): 1754 (acetate C=O); 1650 (conjugated CO); 1220 cm$^{-1}$ (acetate C=O). (Found: C, 56·8; H, 5·0; $OCH_3$, 3·3. $C_{36}H_{36}O_{18}$: requires C, 57·1; H, 4·8; $1OCH_3$, 4·1%.)

*Parkinsonin*-A (70 mg) in abs. MeOH (30 ml) was methylated using diazomethane in ether (48 hr). Recrystallization of the methyl ether from dry acetone afforded pale yellow prisms, m.p., 273–274° (45 mg). It gave no colour with alcoholic $FeCl_3$; $[\alpha]_D^{28}$ +51·1° (7·05 mg, 1 ml pyridine); UV (i) $\lambda_{max}$ 245, 266 and 334 m$\mu$ (4·4, 4·3 and 4·4); IR (KBr disc): 3509 (OH) 1640 (conjugated CO); 1042 cm$^{-1}$

($OCH_3$). (Found: C, 59·3; H, 6·0; $OCH_3$, 22·1. Calc. for $C_{25}H_{28}O_{11}$: C, 59·5; H, 5·6; $4OCH_3$, 24·6%.)

Fission of *Parkinsonin*-A (100 mg) was carried out using HI in phenol as mentioned earlier. Repeated crystallization of the brownish yellow product first from aqueous MeOH and then MeOH gave yellow needles, m.p., >300° (30 mg). It was identical with luteolin in all respects, UV and IR spectra and chromatography. Acetate (pyridine–acetic anhydride) m.p. and mixed m.p. with authentic luteolin tetra-acetate, 222–224°.

*Periodate oxidation.* *Parkinsonin*-A trimethyl ether (2 mg, 0·2 ml water) was oxidized with sodium metaperiodate (2 mg), the dialdehyde reduced with $NaBH_4$ (2 mg), hydrolysed with HCl under conditions mentioned earlier and glycerol identified by chromatography. Glucose was used as a standard. Estimation of the consumption of the number of moles of periodate and of the formic acid liberated was done under conditions mentioned earlier. The trimethyl ether consumed 2·06 moles of periodate with the liberation of 0·98 mole of formic acid.

*Parkinsonin-B*

It was very soluble in $Na_2CO_3$aq and NaOHaq giving deep yellow solutions. It gave a yellow solution with green fluorescence in conc $H_2SO_4$, a green colour with alcoholic $FeCl_3$, a pink colour with Mg and HCl and a positive Molisch's test. UV data: (i) 246, 270 and 350 mμ (3·5, 3·5 and 3·0); (ii) 250, 275 and 363 mμ (3·5, 3·3 and 4·2); (iii) 275, 291 and 350 mμ (3·1, 3·2 and 3·0); (iv) 275, 345 and 370 mμ (4·3, 4·2 and 3·9); (v) 262, 312 and 390 mμ (3·5, 3·5 and 3·3); IR (nujol mull): 3571 (OH); 1639 $cm^{-1}$ (conjugated CO). It did not show appreciable rotation. (Found: C, 55·7; H, 5·0; $OCH_3$, 14·0. $C_{23}H_{24}O_{11}\cdot H_2O$ requires: C, 55·9; H, 4·9; $2OCH_3$, 12·6%.)

*Parkinsonin*-B (100 mg), in a mixture of dioxan (25 ml), EtOH (35 ml) and water (2 ml) was methylated using diazomethane in ether. After 48 hr the solvents were distilled off and the pale yellow residue dried by adding benzene and evaporation. The white amorphous powder thus obtained, crystallized from dry acetone as colourless needles, m.p. 258–259°, undepressed on admixture with epi-orientin tetra-O-methylether; $[\alpha]_D^{28}$ −40·3° (8·35 mg, 1 ml pyridine); UV in EtOH: 246, 266 and 344 mμ (4·4, 4·3 and 4·4); IR (KBr disc); 3509 (OH); 1639 (conjugated CO); 1042 $cm^{-1}$ ($OCH_3$). (Found: C, 55·2; H, 6·0; $OCH_3$, 19·8. $C_{25}H_{28}O_{11}\cdot 2H_2O$ requires: C, 55·5; H, 5·9; $4OCH_3$, 22·9%.)

Since the amount of *Parkinsonin*-B was very small, fission of the mixture of pigments (1 g) was done in phenol (6 g) with HI (10 ml, *d* 1·7) at 135–137° for 7 hr. Repeated crystallization of the brownish yellow product first from aqueous MeOH and then MeOH gave yellow needles, m.p., >300° (370 mg). The aglycone was identical with luteolin in all respects, spectra and chromatography. The acetate of the aglycone (200 mg), on recrystallization from EtOH gave colourless needle (150 mg) m.p. and mixed m.p. with authentic luteolin tetra-acetate, 222–224°. (Found: C, 60·6; H, 4·1. Calc. for $C_{23}H_{18}O_{10}$: C, 60·8; H, 4·0%.)

*Periodate oxidation.* Oxidation of *Parkinsonin*-B dimethyl ether with sodium metaperiodate, reduction of the dialdehyde with $NaBH_4$ and hydrolysis with HCl yielded glycerol, identified by chromatography. The dimethyl ether consumed 2·1 moles of periodate with liberation of 1·08 moles of formic acid.

*Ferric chloride oxidation and the identification of glucose.* The mixture of pigments (1 g) was treated with $FeCl_3$aq (5 g, 16 ml water) yielding a dark green coloured complex which was heated under reflux at 115° for 15 min and then at 125° for 6 hr. The mixture was diluted with water and the dark coloured complex filtered off. The pale yellow filtrate was treated successively with resins IRC-120(H) and IRA-400(OH) to remove Fe and chloride ions and the neutral solution evaporated. The resulting syrup and an authentic sample of glucose had the same chromatographic behaviour ($R_f$ 0·35 in circular paper chromatography and $R_f$ 0·24 in ascending paper chromatography) using (g) as solvent and aniline hydrogen phthalate as the developer. It also formed an osazone, m.p., 208–210° in 10 min.

*Acknowledgement*—We are grateful to Prof. Hörhammer for kindly supplying an authentic sample of orientin.