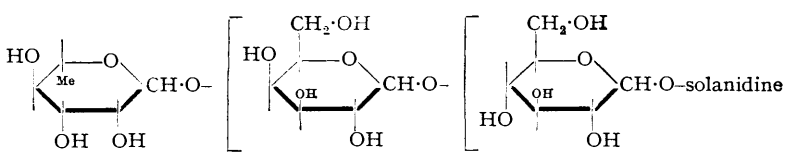
566. Solanum *Alkaloids. Part X.* The Mode of Linkages in the Trisaccharide Moiety of Solanine and Solasonine.*

By Lindsay H. Briggs, and L. C. Vining.

Periodate oxidation of solanine shows that the three sugars, L-rhamnose, D-galactose, and D-glucose, of the trisaccharide moiety are probably linked in the 1, 1 : 4, and 1 : 6, position respectively. Solasonine reacts similarly and must contain the same trisaccharide. The interpretation of the results has been assisted by comparison with the quantitative periodate oxidation of amygdalin. Oxidation of rutin goes beyond the expected values, indicating that the flavonol nucleus is also attacked. 1-Hydroxyanthraquinone is oxidised by periodate to 1 : 4-dihydroxyanthraquinone.

Solanine on hydrolysis affords the aglycone, solanidine, and three sugars, L-rhamnose, D-galactose, and D-glucose (Zwenger and Kind, *Annalen*, 1859, **109**, 244; 1861, **118**, 129; 1865, **123**, 341; Schulz, *Z. Zuckind. Böhm.*, 1900, **25**, 89; Zeisel and Wittmann, *Ber.*, 1903, **36**, 3554; Votoček and Vondraček, *ibid.*, p. 4372; Heiduschla and Sieger, *Arch. Pharm.*, 1917, **255**, 18). Since solanidine is a tertiary base with the only oxygen atom present as a hydroxyl group at $C_{(3)}$ (Prelog and Szpilfogel, *Helv. Chim. Acta*, 1942, **25**, 1306; Uhle and Jacobs, *J. Biol. Chem.*, 1945, **160**, 243) the three sugars must form a trisaccharide combined with this hydroxyl group. From a study of the acetylated glycoside Zemplén and Gerecs (*Ber.*, 1928, **61**, 2294) showed that solanine is constituted in the order of components, rhamnose–galactose–glucose–solanidine. Solanine has no reducing properties so that the three sugars must be linked through their potential aldehyde groups. Solanine is not hydrolysed, as shown by the lack of reducing power towards Fehling's solution, when treated with 0·01N-hydrochloric acid for 7 hr. From this we assume that all the sugars are pyranose (cf. Haworth, *Ber.*, 1932, **65**, *A*, 50) and the following schematic formula illustrates the trisaccharide moiety of solanine.



Neglecting the α- or β-nature of the sugar linkages for the moment it may be seen that there are 16 possible ways of combining the sugars in the order and manner given.

* Part IX, *J.*, 1952, 3591.

Since the discovery by Malaprade (*Compt. rend.*, 1928, **186**, 382) that periodic acid oxidises glycols and triols quantitatively, the method has been used extensively for the elucidation of sugar structures (cf. *Org. Reactions*, 1944, **2**, 341—375). Solasodine, and presumably solanidine, is not oxidised to any appreciable extent by periodic acid but solanine is readily oxidised at 25°.

The number of mols. of periodic acid which would be consumed, and of formic acid produced, by each of the 16 possible combinations of sugars in solanine ranges from 2 and 1 to 6 and 3. Experiment (Fig. 1) shows that 5 mols. of periodic acid are consumed and

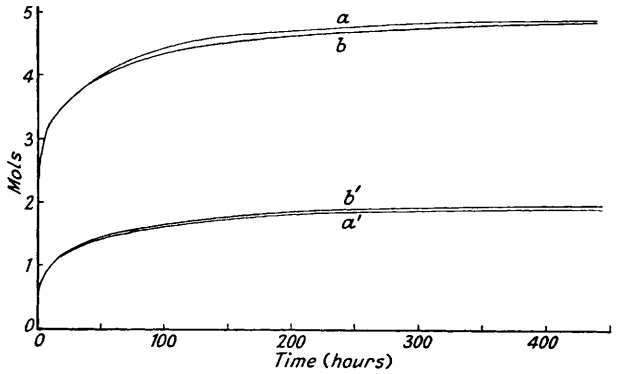
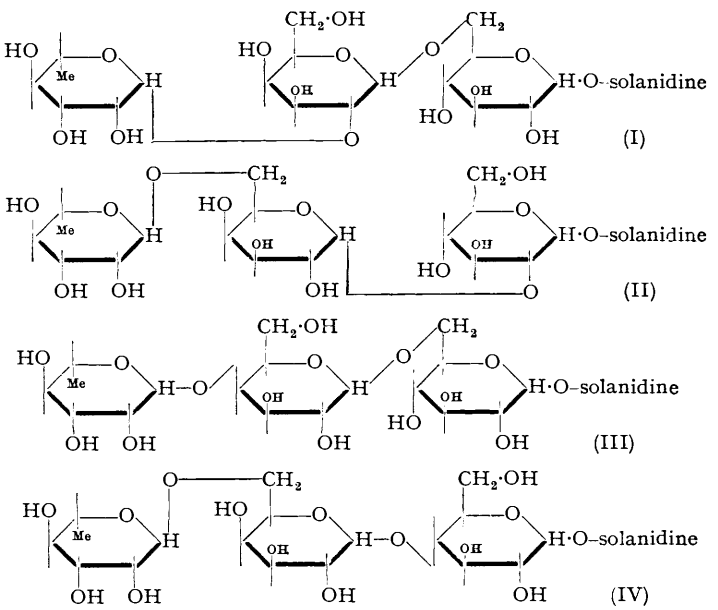


FIG. 1. *Periodate Oxidation of (a) Solanine and (b) Solasonine at* 25°.
Top curves : Periodate consumed.
Lower curves : Formic acid produced.

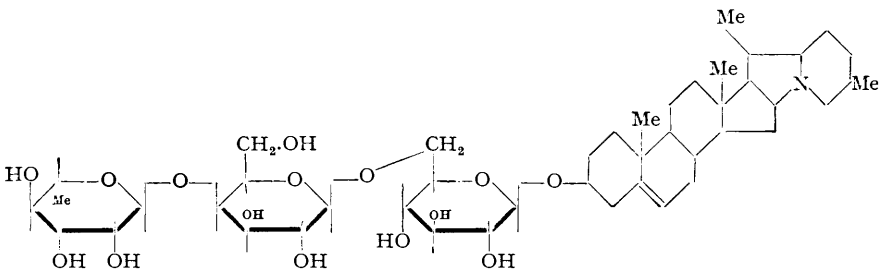
2 mols. of formic acid are produced, limiting the possibilities to four, *viz.*, (I) monosaccharide linkages 1, 1 : 2, 1 : 6, (II) 1, 1 : 6, 1 : 2, (III) 1, 1 : 4, 1 : 6, and (IV) 1, 1 : 6, 1 : 4. Structures involving a 1 : 2′-linkage are extremely rare in Nature, only three examples being recorded [sophorose (2-D-glucosyl β-D-glucoside) (Rabaté, *Bull. Soc. chim.*, 1940, **7**, 565; Freudenberg, Knauber, and Cramer, *Chem. Ber.*, 1951, **84**, 144; Gakhokidze, *J. Gen. Chem. U.S.S.R.*, 1941, **11**, 117); 2-L-rhamnosyl D-galacturonide (Tipson, Christman,



and Levene, *J. Biol. Chem.*, 1939, **128**, 609); apiin (Hemming and Ollis, *Chem. and Ind.*, 1953, 85)]. Although the combinations (I) and (II) with 1 : 2-linkages cannot be excluded at this stage (they will be excluded on later evidence), the most likely structures are (III) and (IV).

Now (III) and (IV) can be differentiated by the rates of reaction at 25°. Both contain the same *cis-trans*-triol of rhamnose and further *trans*-glycols in the galactose and glucose portions severally. In addition, (III) contains a further *trans-trans*-triol whereas (IV) contains another *cis-trans*-triol. Price *et al.* (*J. Amer. Chem. Soc.*, 1938, **60**, 2726; 1942, **64**, 552) have shown that *cis*- and *trans*-glycols react with periodic acid at markedly different rates and the same is presumably true in regard to *cis-trans*- and *trans-trans*-triols. It is certainly true in the case of oxidations with lead tetra-acetate which has a similar action (cf. Freudenberg and Rogers, *ibid.*, 1937, **59**, 1602; Richtmyer, Carr, and Hudson, *ibid.*, 1943, **65**, 1477; Hockett and McClenahan, *ibid.*, 1939, **61**, 1667). In certain triols the consumption of the second mol. of lead tetra-acetate is influenced by the possibility of lactol formation from the hydroxy-dialdehyde formed by the initial action of the first mol. (cf. Helferich, *Ber.*, 1931, **64**, 104; Hockett and McClenahan, *J. Amer. Chem. Soc.*, 1938. **60**. 2061; Hockett, Dienes, and Ramsden, *ibid.*, 1943, **65**, 1474; Hockett, Nickerson, and Reeder, *tert.*, *ibid.*, 1944, **66**, 472). In the present case no α-hydroxy-lactol formation is possible with the combinations (III) and (IV) and, on the assumption that the action of periodate follows that of lead tetra-acetate, the rate of oxidation should be governed mainly by the configuration of the triol groupings.

Since the triol portions of the molecule are responsible for the production of formic acid, it would be expected that in (IV), with two similar triol groupings, the two mols. of formic acid would be produced at approximately the same rate. On the other hand, with (III), which has two different triol groupings, the rates would be expected to differ. Fig. 1 shows clearly that the two mols. of formic acid are produced at greatly differing rates, from which it may be concluded that the combination of sugars in solanine is probably represented by (III). No evidence can be adduced from these experiments relating to the α- or β-linkages of the sugars, but from the ease of hydrolysis of solanine with 2% mineral acids they are all probably β. Solanine may then be tentatively represented by the annexed formula.



Oddo and Caronna's work (*Ber.*, 1934, **67**, 446, and earlier papers) and results recorded in Part II (*J.*, 1942, 3) showed that solasonine also contains a trisaccharide moiety consisting of the same sugars, L-rhamnose, D-galactose, and D-glucose, joined in the same order as in solanine to the hydroxyl group at $C_{(3)}$ of the aglycone, solasodine, represented by a revised formulation in Part V (*J.*, 1950, 3013). Solasonine, like solanine, has no reducing properties, so that the sugars must be joined through their potential aldehyde groups. The sugar linkages are all probably β, from the relative ease of hydrolysis with 2% mineral acids. Solasonine is, however, not hydrolysed, as shown by the lack of reducing power towards Fehling's solution, when treated with 0·01N-hydrochloric acid for 4½ hours, from which we again assume that the sugars are pyranose.

Periodate oxidation of solasonine at 25° afforded results (Fig. 1) practically indistinguishable from those of solanine, so that both probably contain the same trisaccharide. Solasonine may then be represented similarly to solanine. The formula for solasonine given in Part V illustrates the glucose unit with a β-linkage. This was based only on the phytochemical ground that most glucosides have a β-linkage.

Solauricine (Part IV, *J.*, 1942, 17) on hydrolysis affords the aglycone solauricidine, possibly an epimer of solasodine about $C_{(22)}$ (Part V, *loc. cit.*), and the same three sugars as above. It is most likely that the trisaccharide moiety is the same as that of solanine

and solasonine and, in our opinion, therefore, solauricine is identical with solasonine, apart from the epimerisation at $C_{(22)}$.

The trisaccharide discussed in this paper is found only in members of the *Solanum* species and the name, solanose, is proposed for it. A different trisaccharide, L-rhamnose-L-rhamnose-D-glucose, occurs combined with solasodine in solamargine, from *Solanum marginatum* (Part VIII, *J.*, 1952, 3587, and forthcoming communication).

Further support for the structure of the trisaccharide was sought by oxidation and hydrolysis of the intermediate hexa-aldehyde obtained by periodate oxidation, and identification of the final fragments, by the procedure developed and applied by Hudson and his co-workers (cf. Richtmyer and Hudson, *J. Org. Chem.*, 1946, **11**, 610). Either of the structures, (III) or (IV), for solanine (or corresponding formulæ for solasonine) should, on complete periodate oxidation, give rise to a hexa-aldehyde capable of oxidation to the hexacarboxylic acid with bromine water. Hydrolysis of the hexa-acid should then give

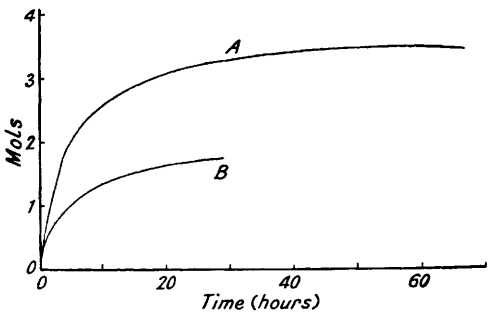
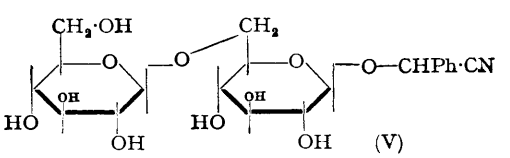


FIG. 2. *Periodate oxidation of amygdalin at 25°.*
A, Periodate consumed.
B, Formic acid produced.

four fragments, identical in three cases (L-lactic, glyoxylic, and D-glyceric acid) but differing in the fourth component, D-threonic acid arising from (III) and the epimeric D-erythronic acid from (IV).

Structures for solanine and solasonine derived from combinations (I) and (II), containing 1 : 2′-linkages, could not by the same procedure yield either threonic or erythronic acid but would give a hydroxymalonic acid in addition to the other three acids mentioned above.

By systematic analysis of the final mixture of acids through their sparingly soluble metal salts (for details, see Experimental section) we have identified glyceric acid (as calcium D-glycerate) and glyoxylic acid (as strontium oxalate after further oxidation to oxalic acid with bromine water), but insufficient lactic acid was recovered (as its zinc salt) for identification. The most significant fact, however, was the isolation of an acid, as its brucine salt, m. p. 210—212, $[\alpha]_D^{16}$ −31°. The brucine salts of D-threonic and D-erythronic acids have very similar properties, m. p. 212°, $[\alpha]_D^{20}$ −29·4° (Isbell, *J. Res. Nat. Bur. Stand.*, 1942, **29**, 227) and m. p. 212°, $[\alpha]_D^{20}$ −23° (Glattfield, *J. Amer. Chem. Soc.*, 1928, **50**, 149), respectively, and although the properties of our isolated product do not



(V)

exclude its identification as the salt of erythronic acid, they lie somewhat closer to those of threonic acid and thus support the probable structure for solanine and solasonine based on (III). It does, however, definitely exclude a structure based on 1 : 2-linkages as in (I) and (II).

As a further contribution to the relation of configuration to the rate of periodate oxidation we have oxidised amygdalin and rutin.

Amygdalin (V), which has its two glucose portions linked in the 1 : 1- and 6-positions (Campbell and Haworth, *J.*, 1924, 1337) was oxidised with periodate by Courtois and

Valentino (*Bull. Soc. Chim. biol.*, 1944, **26**, 469) and Halsall, Hirst, and Jones (*J.*, 1947, 1431). The former found that 2 mols. of formic acid were produced and 4 mols. of periodate consumed, the latter that 2 mols. of formic acid were produced, both results agreeing with the predicted values. We have confirmed these results in carrying out the experiment quantitatively at 25° (Fig. 2). The results differ, however, from those with solanine and solasodine. There is no rapid initial consumption of periodate or production of formic acid, which harmonises with the fact that amygdalin, unlike solanine and solasonine, contains no *cis*-groups. The action of periodate is thus similar to that of lead tetra-acetate in its action on triols (cf. Hockett, Dienes, and Ramsden, *loc. cit.*).

Rutin, a rhamnoglucoside of quercetin (Zemplén and Gerecs, *Ber.*, 1935, **68**, 1318), has its sugar moiety linked in the 1 : 1′- and 6′-positions respectively. Periodate oxidation of this compound with both a *cis-trans*- and a *trans-trans*-triol would have provided further useful information on the rates of reaction, simulating better the structures of solanine and solasonine. Preliminary experiments showed, however, that the consumption of periodate and production of formic acid went far beyond the expected 4 and 2 mols. respectively. It appears that the flavonol nucleus also underwent oxidation. Pennington and Ritter (*J. Amer. Chem. Soc.*, 1947, **69**, 187) observed the same behaviour with other substituted phenols.

Morindin, a rhamnoglucoside of morindone (1 : 5 : 6-trihydroxy-2-methylanthraquinone; Briggs and Dacre, *J.*, 1948, 564) could also provide additional evidence, but again preliminary investigations showed that 1-hydroxyanthraquinone could be oxidised by periodate to 1 : 4-dihydroxyanthraquinone and other yet unidentified products in small yield. Quercetin 3 : 7 : 3′ : 4′-tetramethyl ether, however, was not attacked by periodate.

Experimental

Preliminary experiments were made to determine titratable formic acid in the presence of the aglycones, solanidine and solasodine, and the approximate quantity of periodate consumed and the formic acid produced in the oxidation of both solanine and solasonine before making quantitative measurements at 25°.

Determination of Formic Acid consumed by Solanine and Solasonine.—Formic acid (50 c.c. of 0·0075N) was added to two weighed samples of dried solasonine [(*a*) 0·2069, (*b*) 0·2055 g.], and each solution made up to 100 c.c. When dissolution was complete, 10-c.c. aliquots were removed and the amount of formic acid was determined by the addition of potassium iodide and estimation of the liberated iodine with 0·01N-sodium thiosulphate. Formic acid consumed per mol. of solasonine was (*a*) 0·981 and (*b*) 0·993 mol.

Similarly, solanine (0·0764 g.) was dissolved in formic acid (15 c.c. of 0·0112N), the solution made up to 100 c.c., and the amount of free formic acid in 25-c.c. aliquots determined. Formic acid consumed per mol. of solanine, 0·982 mol.

Rate of Oxidation of Solanine by Sodium Metaperiodate at 25°.—Finely powdered, dried solanine (0·3865 g.) was dissolved in formic acid [50 c.c.; 0·0112N (1·36 mols.)] and distilled water (100 c.c.). Sodium metaperiodate solution [70 c.c.; 0·305N (6·49 mols.) (Hill, *J. Amer. Chem. Soc.*, 1928, **50**, 2678), standardised by adding potassium iodide and sulphuric acid and titrating the liberated iodine with sodium thiosulphate solution] was added and the whole diluted to 500 c.c., shaken, and placed in a thermostat at 25°. All the reactants had been previously kept at 25° for ½ hr. Aliquot portions were removed at selected intervals (based on the preliminary experiments), and the periodate consumed and formic acid produced were determined as follows.

For the determination of periodate the reaction was stopped in aliquot samples (10 c.c.) by adding 20% potassium iodide solution (2 c.c.) and 2N-sulphuric acid (10 c.c.), and the liberated iodine titrated with 0·01N-sodium thiosulphate (starch). Since periodate liberates 4 mols. of iodine from acidified potassium iodide while iodate liberates only 3 mols., any difference in titre can be used to calculate the amount of periodate consumed.

For the determination of formic acid aliquot portions (25 c.c.) were removed and the oxidation stopped by adding excess (1 c.c.) of ethylene glycol, purified by distillation from solid potassium hydroxide and neutral to methyl-red. After 5 min., 1 c.c. of 20% potassium iodide solution was added and, after a further 5 min., the liberated iodine was titrated against 0·01N-sodium thiosulphate (starch). The results are illustrated in Fig. 1.

Rate of Oxidation of Solasonine by Sodium Metaperiodate at 25°.—Finely powdered dried solasonine (0·4300 g.) was dissolved in 0·0112N-formic acid (50 c.c., 1·25 mols.) and distilled water (100 c.c.). 0·305N-Sodium metaperiodate (70 c.c., 5·93 mols.) was added and the volume made up to 500 c.c. The above procedure was again followed with the results also illustrated in Fig. 1.

Degradation of the Sugar Moiety of Solasonine to Simple Acids.—Solasonine (4 g., 1 mol.) was added to a solution of sodium metaperiodate (5·4 g., 6 mols.) in water (400 c.c.) and the mixture set aside. The solasonine had dissolved completely within 5 hr. At the end of 3 weeks the solution became brown owing to the separation of inorganic material (350 mg.) which was filtered off. The colourless filtrate was acidified with 90% formic acid (1·2 g., 5·5 mols.), titrated with hot 10% strontium hydroxide solution (14 c.c.) (methyl-red), and set aside for several days at 0°. The white crystalline precipitate (5·1 g.), presumably a mixture of strontium periodate and iodate, was then filtered off. Concentrated hydrobromic acid (1 c.c.) was added and then bromine (9·5 g., 13 mols.), dropwise. Decolorisation occurred at first with evolution of gas and, on shaking, a curdy orange precipitate separated. The rotation of the solution became constant after 5 days, whereafter excess of bromine was removed by aeration for 10 hr. Hydrolysis was then brought about by heating at 100° for 20 hr., most of the precipitate dissolving. The remaining black residue (300 mg.) was removed and bromine (2 g., 3·1 mols.) again added to oxidise the expected glyoxylic acid to oxalic acid. A pale orange precipitate was again formed and after 2 days excess of bromine was removed as before. A black tar was filtered off. The clear solution was neutralised until alkaline to methyl-red but still just acid to phenolphthalein by the addition of solid strontium hydroxide (5 g.) and finally titration with hot 10% strontium hydroxide solution (13 c.c.). After several hours at 0° the pale brown precipitate was filtered off (fraction A; 900 mg.).

A sample of fraction A in hydrochloric acid gave an alkaloid test with Dragendorff's reagent. The whole fraction was therefore warmed with alcohol (50 c.c.), and the colourless residue (200 mg.) filtered off. This was dissolved in hot dilute hydrochloric acid and treated with dilute sulphuric acid until no more strontium sulphate was precipitated. The solution was then concentrated to a small volume. Colourless needles of oxalic acid dihydrate (100 mg.) separated, having m. p. and mixed m. p. 102°.

The filtrate from fraction A was freed from strontium ions by adding dilute sulphuric acid until precipitation was just complete, and filtering. After concentration in a vacuum to *ca.* 50 c.c. the filtrate was neutralised to methyl-red with zinc oxide (*ca.* 4 g.), and a dark brown residue filtered off from the hot solution. No zinc lactate could be detected in this residue which was almost completely soluble in alcohol. The solution remained clear on cooling, so it was again acidified with hydrobromic acid, concentrated to half-volume, and neutralised with zinc oxide. Brown resin was removed from the hot solution and on cooling to 0° a grey amorphous precipitate was removed (fraction B; 290 mg.).

Fraction B could not be completely redissolved in 5 c.c. of hot water. The insoluble portion was filtered off and the filtrate concentrated to a small volume. The colourless needles which separated (21 mg.) had $[\alpha]_D^{16}$ −4·9° [l = 1 (micro-tube); c 2·1 in H_2O) and were perhaps zinc lactate (Maclay, Hann, and Hudson, *J. Amer. Chem. Soc.*, 1939, **61**, 1660, record $[\alpha]_D^{20}$ −7·7° for zinc L-lactate) but attempts to confirm this by the preparation of brucine lactate were unsuccessful.

The filtrate from fraction B was freed from zinc ions by the passage of hydrogen sulphide at a pH kept above 3 by the addition of sodium hydroxide solution, and separation of the zinc sulphide. Tests with Dragendorff's reagent at this stage still indicated the presence of alkaloid. Calcium bromide (5 g.) was added and then all bromide ions were removed by adding excess of silver sulphate solution and filtering. Sulphate ions were next removed by a sufficiency (no excess) of hot barium hydroxide solution, and silver ions by hydrogen sulphide. Excess of hydrogen sulphide was removed by aeration for 3 hr., and the solution concentrated in a vacuum to *ca.* 20 c.c. and just neutralised to phenolphthalein with calcium oxide. After the addition of alcohol (100 c.c.) a colourless amorphous precipitate was filtered off (fraction C; 300 mg.) and dissolved in hot water and the solution concentrated. Colourless needles separated on cooling (fraction D; 50 mg.), and after two recrystallisations from water had m. p. 138°, $[\alpha]_D^{16}$ +14·8° [l = 1 (micro-tube); c 3·1 in H_2O]. Jackson and Hudson (*J. Org. Chem.*, 1946, **11**, 614) record m. p. *ca.* 142°, $[\alpha]_D^{20}$ +15·5°, for calcium D-glycerate dihydrate.

The filtrate from fraction C was taken to dryness in a vacuum, yielding a white powder (150 mg.). This was dissolved in water (2 c.c.), dilute oxalic acid solution added until precipitation was complete, and the calcium oxalate filtered off. Alcohol (5 c.c.) and brucine

(0·8 g., 4 mols.) were added to the filtrate, and the mixture was heated at 100° for 3 hr. Excess of brucine was then removed with chloroform, and the solution concentrated in a vacuum to a thick syrup. This was finally dissolved in hot alcohol (2 c.c.) and allowed to crystallise. The colourless needles which separated (12 mg.), after repeated crystallisation from alcohol, had m. p. 210—212°, $[\alpha]_D^{16}$ −31° [l = 1 (micro-tube); c 1 in H_2O]. Isbell (*J. Res. Nat. Bur. Stand.*, 1942, **29**, 227) records m. p. 212°, $[\alpha]_D^{20}$ −29·4°, for brucine D-threonate.

Rate of Oxidation of Amygdalin by Sodium Metaperiodate at 25°.—Amygdalin (0·1803 g.) was dissolved in water (100 c.c.), 0·305N-sodium metaperiodate (50 c.c.) added, and the mixture made up to 500 c.c., shaken well, and placed in a thermostat at 25°. The reactants had previously been kept at 25° for ½ hr. The periodate and formic acid were determined at intervals, selected from preliminary experiments; results are recorded graphically in Fig. 2.

Oxidation of 1-*Hydroxyanthraquinone with Sodium Metaperiodate.*—In a preliminary experiment 1-hydroxyanthraquinone (0·0314 g.; purified by chromatography on freshly calcined magnesia) was dissolved in an aqueous solution of sodium hydroxide (0·5 g. in 300 c.c.), 0·308N-sodium metaperiodate [(15 c.c., 4·25 mols.) added, and the mixture made up to 500 c.c. The amount of periodate consumed was determined at intervals in the usual way [Found: periodate per mol. of 1-hydroxyanthraquinone: 1·1 (1 hr.), 1·76 (2 hr.), 2·19 (3 hr.), 3·88 (14·5 hr.), 4·25 mols. (26·5 hr.)].

In an experiment to isolate the oxidation products chromatographically, pure 1-hydroxyanthraquinone (1 g.) in sodium hydroxide solution (5 g. in 2 l.) was allowed to react with sodium metaperiodate solution (1 g. in 50 c.c.; 1 mol.) for 3 days. In this time a dark brown solid had precipitated which was filtered off, acidified with dilute hydrochloric acid, dried (yield, 210 mg.), dissolved in acetone, and chromatographed on magnesia. The main solution was acidified and set aside for some days. A similar precipitate formed which was also treated as above (yield, 1·1 g.). The chromatograms and the colour of the products obtained from similar bands appeared identical in each case, so the products were combined. Systematic chromatography of the material and development of the bands with acetone and acetone containing acetic acid (cf. Briggs and Nicholls, *J.*, 1949, 1241) afforded five major bands: dark blue, brown, mauve, red, and salmon. The distinct bands were decomposed with dilute hydrochloric acid, and the liberated anthraquinones filtered off. The respective products were brown (5 mg.), orange (85 mg.), orange-red (17 mg.), yellow (530 mg.), and yellow (250 mg.).

Despite the distinct difference in colour of the final two bands the material recovered from each proved to be unchanged 1-hydroxyanthraquinone (yellow needles, m. p. and mixed m. p. 196—196·5°, after crystallisation from alcohol).

The material from the mauve band was purified by sublimation at 120—150°/0·05 mm. The m. p. of the product, 188°, was not depressed by an authentic specimen of 1 : 4-dihydroxyanthraquinone while its colour reactions and its half-wave potential (−0·788 v) were also identical.

Unfortunately most of the material from the brown band was lost and there was insufficient material from this and the first dark blue band for identification.

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