

## Structures of Isamic Acid and Methylisatoid

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Isamic acid, a red crystalline product from the reaction of isatin with ammonia, is proved to be 1,2-dihydro-2-oxo-spiro-[3*H*-indole-3,2'(1'*H*)-quinazoline]-4'-carboxylic acid, as suggested by G. F. Field. The colour and lack of basicity appear to be due in part to spiroconjugation. Methylisatoid, from *O*-methylisatin and moist air or aqueous acetic acid, is shown by rational synthesis and degradation to be methyl 6,12-dihydro-12-hydroxy-6-oxoindolo-[2,1-*b*]quinazoline-12-carboxylate.

ISAMIC ACID (imasatinic acid) and its amide (amasatin, isamide) were separated in 1842 by Laurent<sup>1,2</sup> from the mixture of products he obtained by the action of ammonia on isatin. The constitutions of these two substances were studied by Sommaruga and Reichardt,<sup>3</sup> Reissert and Hoppmann,<sup>4</sup> and Jacini;<sup>5</sup> but it was not until 1967 that de Mayo and Ryan<sup>6,7</sup> succeeded in showing that isamic acid, as usually prepared, is a monohydrate, and they suggested the first structures based on the correct molecular formula C<sub>16</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>.

Isamic acid has normal acidity for a carboxylic acid and it is a very weak base, though it forms violet solutions in strong mineral acids. It is stable in boiling dilute sodium hydroxide and can be prepared by alkaline hydrolysis of its amide or of its methyl ester. On the other hand, it is hydrolysed easily, and quantitatively,<sup>7</sup> by dilute mineral acid to isatin (2 mol. equiv.) and ammonia (1 mol. equiv.).

de Mayo and Ryan<sup>7</sup> obtained good evidence that isamic acid retains one oxindole ring of the two isatin molecules that enter into its composition. Thus the n.m.r. spectrum of the methyl ester shows a one-proton peak at  $\delta$  9.1 that disappears on addition of deuterium oxide; an easily prepared monoacetyl derivative of the ester had the i.r. spectrum ( $\nu_{\text{max}}$  1770 and 1720 cm<sup>-1</sup>) of a diacylimide and the acetyl signal in the n.m.r. spectrum was at  $\delta$  2.48; this acetyl derivative easily added one molecule of methanol with generation of a

new ester group (opening of an *N*-acetyl-lactam ring). All these properties are rather characteristic of oxindoles. Much further evidence leading to the same conclusion was obtained by study of the hydrogenation products of methyl isamate; for this the original paper may be consulted.<sup>7</sup>

Isamic acid can be made (*a*) from isatin and ammonia, in poor yield; <sup>1</sup> (*b*) from ammonium isatinate solutions by evaporation; <sup>2</sup> (*c*) from isatin and ammonium acetate; <sup>4</sup> and (*d*) from isatin-3-imide and sodium isatinate.<sup>4</sup> de Mayo and Ryan<sup>7</sup> made an *N*-methylisamic acid from 1-methylisatin-3-imide and sodium isatinate, and showed that its methyl ester still contained one hydrogen atom exchangeable with deuterium. Impressed by the low basicity and deep colour of isamic acid, they interpreted their experimental findings in terms of the structures (I) (*a* or *b*). These they called 'ylids' although, given the two exchangeable hydrogen atoms, they are simply tautomers of 1-(oxindol-3-yl)indazole-3-carboxylic acid (II), a substance which should have an entirely different set of properties. Further, the formulation (I) implies both the formation and the cleavage of the N-N bonds in unexampled conditions.

G. F. Field<sup>8</sup> suggested that the low basicity and the light absorption in the visible region could both be explained if two of the nitrogen atoms in isamic acid were in a 1,2-dihydroquinazoline ring, and he proposed the spiran structure (III) for isamic acid, without adducing

<sup>1</sup> A. Laurent, *J. prakt. Chem.*, 1842, [2] 25, 456.

<sup>2</sup> A. Laurent, *J. prakt. Chem.*, 1845, [2] 35, 108.

<sup>3</sup> E. Sommaruga and E. Reichardt, *Ber.*, 1877, 10, 432.

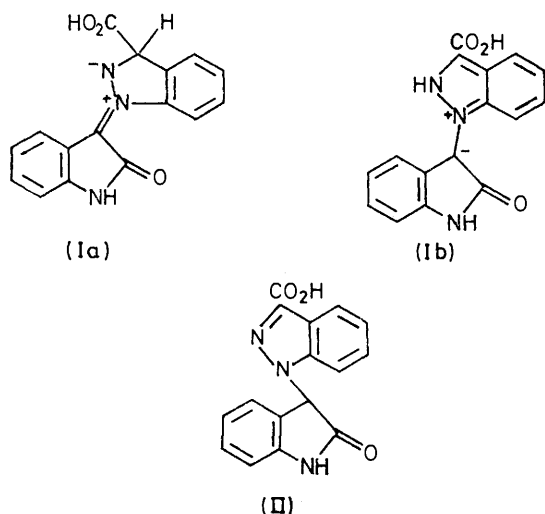
<sup>4</sup> A. Reissert and H. Hoppmann, *Ber.*, 1942, 57, 972.

<sup>5</sup> G. Jacini, *Gazzetta*, 1941, 71, 532; 1942, 72, 510; 1943, 73, 85, 306; 1944, 74, 3; 1947, 77, 295.

<sup>6</sup> P. de Mayo and J. J. Ryan, *Chem. Comm.*, 1967, 88.

<sup>7</sup> P. de Mayo and J. J. Ryan, *Canad. J. Chem.*, 1967, 45, 2177.

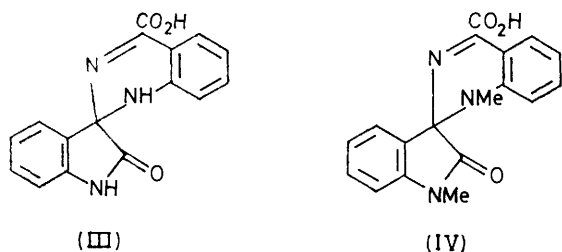
experimental evidence except for the demonstration (already implicit<sup>7</sup>) that methyl isamate contains more than one exchangeable hydrogen atom.



Structure (III) is now proved correct by the following evidence.

(1) *Both exchangeable hydrogens of methyl isamate are on aromatic NH groups.* de Mayo and Ryan,<sup>7</sup> apparently under the impression that Reissert and Hoppmann<sup>4</sup> reported failure to obtain an isamic acid from *N*-methylisatin and ammonia, did not try this reaction. In fact, *N*-methylisatin when boiled with aqueous alcoholic ammonium acetate yields an isamic acid (IV) typical in its colour, spectra, low basicity, and quantitative hydrolysis by acid to *N*-methylisatin and ammonia. Like isamic acid and methylisamic acid,<sup>7</sup> it is a monohydrate and yields an anhydrous methyl ester.

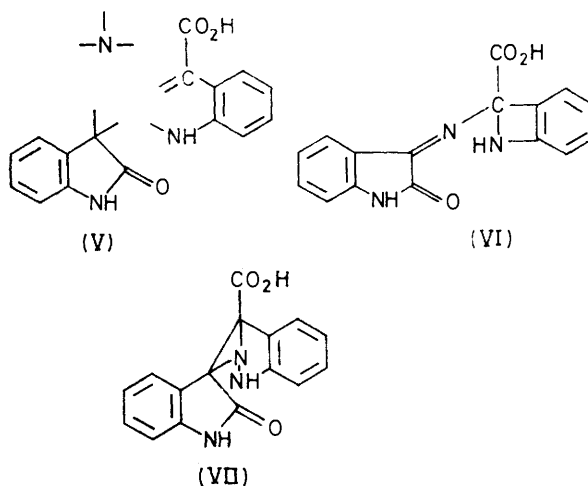
(2) *Isamic acid is a chiral molecule of reasonable but limited optical stability.* Crystallization of brucine isamate from aqueous propan-2-ol afforded yellow prisms. Further amounts were obtained by boiling down mother liquors, until the total yield of this apparently pure diastereoisomeric salt was 64% (128% of



'theoretical'). Decomposition by ammonia gave an ammonium salt that was laevorotatory in aqueous solution and dextrorotatory in 80% methanol. When the ammonium salt was acidified immediately after preparation, (+)-isamic acid of  $[\alpha]_D +90^\circ$  in methanol was obtained. When the aqueous solution of the salt was left at ambient temperature the optical activity declined by *ca.* 5% a day, and racemic isamic acid could be recovered by acidification after 2 months. The acid

itself lost optical activity rather faster (*ca.* 8% a day) in methanol.

Isamic acid has the constitution  $C_{16}H_{11}N_3O_3$ . The carbon is all derived from two molecules of isatin; the mild and various conditions of formation from, and reversion to, isatin appear to rule out skeletal rearrangement. Isamic acid contains one carboxy-group, as shown by the acidity and by the formation and alkaline hydrolysis of a typical ester and amide.<sup>4,7</sup> It also contains an oxindole ring, as is well attested by the evidence<sup>7</sup> cited earlier. If we remember that there are two aromatic NH groups, the problem resolves itself into the correct assembly of the three elements pictured in (V) to form a chiral molecule.

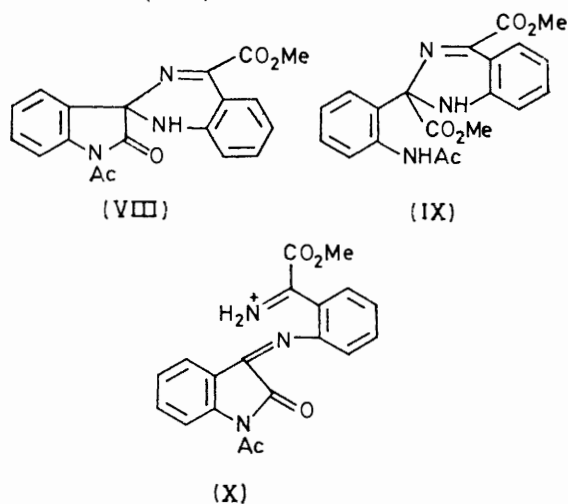


No more than three structures can be so constructed: (VI), (VII), and (III). Of these, (VI) and (VII) can be excluded (apart from the improbability of such strained structures being formed in conditions so mild) on spectroscopic and chemical grounds. Compound (VII) should show no visible colour, and its quantitative decomposition by dilute acid to two molecules of isatin is inconceivable. Structure (VI) is better chemically but it is an isatin-3-imide and would have the u.v. absorption of its class, including bands near 250 and 300 nm. Isamic acid and its *N*-methyl derivatives, and their esters, show no maxima between 400 and 240 nm. Isamic acid, therefore, ineluctably has structure (III).

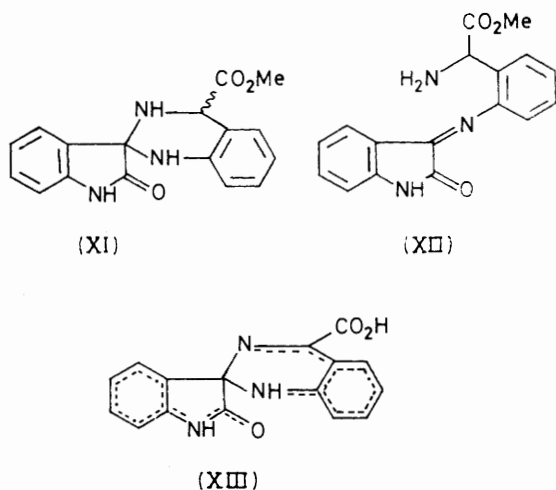
Field<sup>8</sup> indicated that the light absorption and the low basicity of isamic acid are adequately explained by its formulation as a 1,2-dihydroquinazolin-3(1H)-one having two carbonyl groups adjacent to the basic centre. A test of this is provided by comparison of the two substances methyl 1-acetylisamate (VIII) and its methanolysis product (IX).<sup>7</sup> The electronic absorption spectra of these two substances were compared in neutral (tetrahydrofuran) and strongly acidic (8*N*-hydrochloric acid) solution. The substance (VIII) absorbed at longer wavelengths ( $\Delta\lambda_{\max}$  20–36 nm) in both solvents, and the long-wave peak was less intense and broader. The basic strength ( $pK'$ ) of (VIII) and (IX) could be determined

<sup>8</sup> G. F. Field, *Chem. Comm.*, 1969, 886.

by Hammett's<sup>9</sup> procedure for weak bases, though neither substance is stable in acid and measurements were therefore rapid and not highly accurate. Here again the two substances differed;  $pK'$  +0.03 for (IX) and -0.46 for (VIII).



That the protonated forms of (VIII) and (IX) retain an intact dihydroquinazolinone ring and are not tautomers [*e.g.* (X)] was indicated by an experiment with (+)-isamic acid, which also gives an immediate deep violet solution in strong acid. A sample dissolved in 8*N*-hydrochloric acid and neutralized after 30 s retained over 80% of the original optical activity. It seems likely that the spontaneous racemization of isamic acid and of its ammonium salt are due to the formation of a chain tautomer [*e.g.* deprotonated (X)], but this process is obviously slow. The two diastereoisomers<sup>7</sup> of methyl dihydroisamate [reformulated<sup>8</sup> as (XI)]



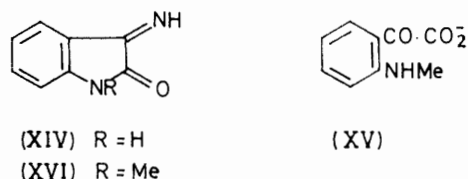
clearly undergo ring-chain tautomerism with greater ease, shown in their interconversion *via* a yellow substance that is presumably (XII).

<sup>9</sup> L. P. Hammett and M. A. Paul, *J. Amer. Chem. Soc.*, 1934, **56**, 827.

<sup>10</sup> H. E. Simmons and T. Fukunaga, *J. Amer. Chem. Soc.*, 1967, **89**, 5208.

Thus (VIII) is significantly more deeply coloured and less basic than the simple dihydroquinazolinone (IX). These differences are both attributable to the electronic delocalization that is called spiroconjugation.<sup>10</sup> The structure (III) for isamic acid qualifies for this effect, since two  $\pi$ -systems surround a common  $sp^3$  carbon atom, as indicated in (XIII). The lower basicity, and the lower frequency and intensity of the longest-wave absorption, in (VIII) as compared with the non-spiran (IX), are all consistent with this explanation.<sup>10</sup>

Most of the chemistry of isamic acid, including the rearrangements observed on oxidation and photolysis (*vide de Mayo and Ryan*,<sup>7</sup> and references cited therein) can be explained without effort on the basis of structure (III); and except for compounds (III), (VIII), (IX), and (XI) no structural reassignment is required. One curious point remains, however: why is isamic acid, rather than its amide, formed from isatin and ammonium acetate? It seems to have been tacitly assumed that the general route to isamic acid proceeds by concurrent formation of isatin-3-imide and an isatinic acid salt, which are known<sup>4</sup> to combine easily to yield isamic acid: the formation of (III) in this reaction is easily understood. de Mayo and Ryan<sup>7</sup> failed, however, to obtain a methylisamic acid by condensation of isatin-3-imide (XIV) with sodium *N*-methylisatinate (XV).



This makes it seem unlikely that 1-methylisatin-3-imide (XVI) and a salt of *N*-methylisatinic acid (XV) react with each other in the successful synthesis of dimethylisamic acid from *N*-methylisatin and ammonium acetate. Further, the hydrolysis of isatin to isatinic acid salts requires rather strongly basic conditions and it is rapidly reversed on acidification, whereas the ammonium acetate synthesis succeeds in a solution that becomes progressively more acidic as acetic acid is formed. Finally, ammonium acetate tends to be an aminating rather than a hydrolysing agent, yet isamide is not formed at all. It seems likely that in these conditions the carboxy-group of isamic acid is formed as an anion by displacement, as in the hypothetical sequence (XVII)  $\rightarrow$  (XIX). An analogy is available in the formation of the thiazolinecarboxylic acid (XXI), rather than the methyl ester or thiolacid, from the oxazolone (XX) and hydrogen sulphide in absolute methanol.<sup>11</sup>

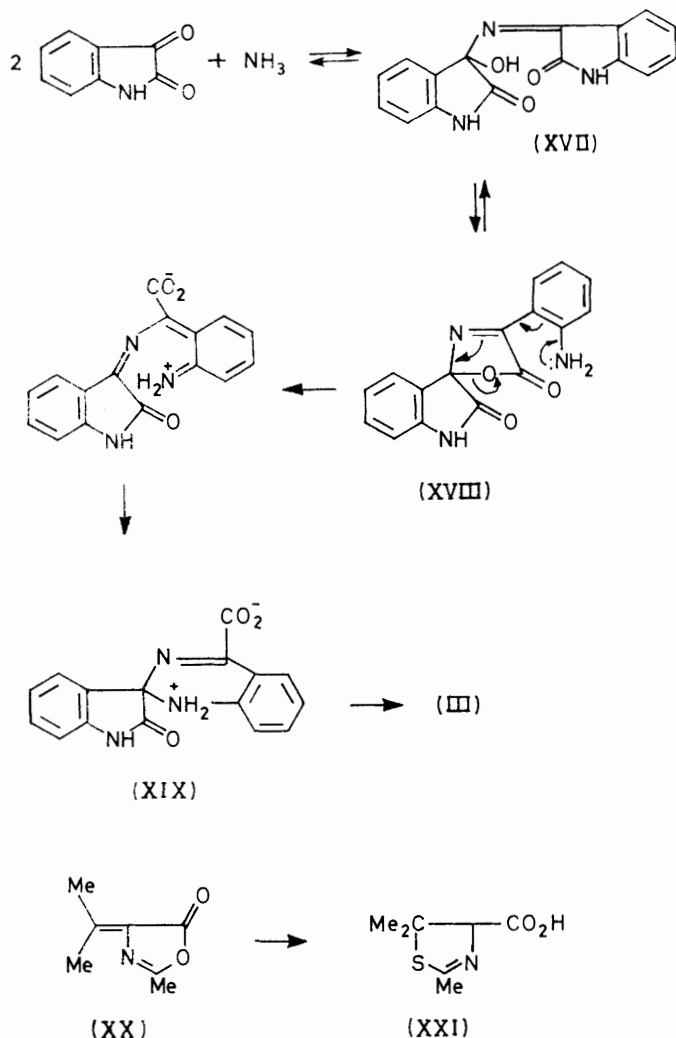
Methylisatoid, a substance first noted<sup>12</sup> as a product of exposure of solid *O*-methylisatin (XXII) to moist air, is made more conveniently<sup>13</sup> by dissolution of (XXII) in acetic acid containing some water. The constitution

<sup>11</sup> W. R. Boon, H. C. Carrington, *et al.*, 'The Chemistry of Penicillin,' Princeton University Press, 1949, pp. 737 and 791.

<sup>12</sup> A. v. Baeyer and S. Oekonomides, *Ber.*, 1883, **15**, 2093.

<sup>13</sup> A. Hantzsch, *Ber.*, 1922, **55**, 3180.

of this rather ill-characterized substance engendered a long unfruitful controversy between Heller and



Hantzsch; a bibliography is given by Bird,<sup>14</sup> who reopened the subject in 1963 and supported one of Hantzsch's suggestions (XXIII) for the structure.

If structure (XXIII) were correct, methylisatoid would be unique as a substance having one oxygen and two nitrogen atoms attached to the same tetrahedral carbon atom. It seemed more reasonable to suppose that *O*-methylisatin is behaving here as many imino-esters do, for example in the formation of 'imino-hydrins':<sup>15</sup> hydrolysis yields an ester and an amine [here combined in the same molecule (XXIV)] which reacts with another molecule of imino-ester to form an amidine (XXV). The preferred tautomeric form of this amidine should be the carbinolamine (XXVI), which is now shown to be the correct structure for methylisatoid.

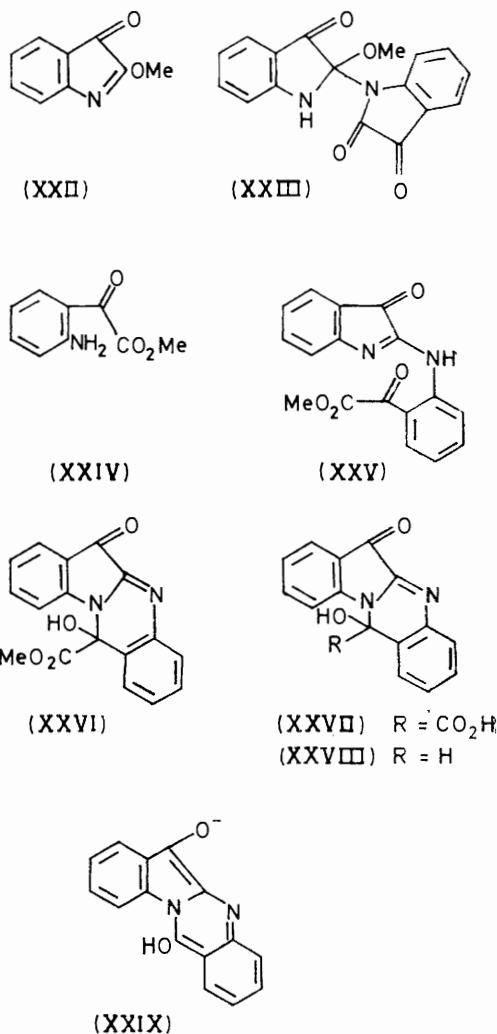
When methylisatoid was heated with aqueous methanolic sodium hydrogen carbonate, careful acidification of the deep red solution gave a hydrogen carbonate-

<sup>14</sup> C. W. Bird, *Tetrahedron*, 1963, **19**, 901.

<sup>15</sup> H. G. Rule, *J. Chem. Soc.*, 1918, **113**, 3.

soluble acid having a complex u.v. spectrum almost identical with that of methylisatoid and an i.r. spectrum also very similar but having, in the C=O stretching region, a band at 1710  $\text{cm}^{-1}$  (which the spectrum of methylisatoid does not show) and no band at 1763  $\text{cm}^{-1}$  (which it does show). The formulation of this substance as the carboxylic acid (XXVII), of which methylisatoid is the methyl ester, was confirmed in three ways.

(i) The acid on gentle warming in solution (acetone, ethyl acetate, or dimethyl sulphoxide) lost carbon dioxide and afforded quantitatively a neutral substance that also resembled methylisatoid in its spectra. It presumably has the same structure as a compound obtained by Bird<sup>14</sup> by acidic hydrolysis of methylisatoid and from *O*-methylisatin and *o*-aminobenzaldehyde; Bird's product melted at a lower temperature but other properties correspond. It is formulated as (XXVIII), following Bird.



(ii) Condensation of *O*-methylisatin and potassium isatin in dimethylformamide containing 1 equiv. of acetic acid gave in high yield an acid identical with that from the hydrolysis of methylisatoid.

(iii) The silver salt of the acid (XXVII) gave methylisatoid on treatment with methyl iodide.

The presence of a methoxycarbonyl group in methylisatoid is thus fully established: the high stretching frequency for the ester carbonyl is attributable to electronegative substitution at the  $\alpha$ -carbon atom, as is also the appreciable acidity of the tertiary hydroxy-group (methylisatoid dissolves in cold dilute sodium hydroxide<sup>14</sup> and can be precipitated by carbon dioxide from the fresh solution). The ease of decarboxylation is consistent with the formation, from (XXVII), of the relatively stable anion (XXIX).

The rational synthesis of methylisatoid from potassium isatinatate and *O*-methylisatin *via* the carboxylic acid (XXVII) appears to allow no structure for methylisatoid other than (XXVI) or a tautomer. No evidence for the presence of a third carbonyl group in methylisatoid [such as is present in the tautomer (XXV)], or in the carboxylic acid, could be found. The difficulties of purifying methylisatoid, and the tendency for some of its properties to vary according to the method of preparation or purification, are not uncommonly encountered with carbinolamines. Its formation from crystalline *O*-methylisatin on exposure to moist air may be a 'non-photochemical reaction in the solid state', as Bird<sup>14</sup> suggested; but *O*-methylisatin is appreciably volatile at room temperature and its initial reaction with water vapour is not necessarily a heterogeneous process.

#### EXPERIMENTAL

I.r. spectra were recorded for paraffin pastes and u.v. spectra for methanolic solutions, except where indicated otherwise.

*1,2-Dihydro-1,1'-dimethyl-2-oxospiro-[3H-indole-3,2'(1'H)-quinazoline]-4'-carboxylic Acid*.—1-Methylisatin (3.24 g), ammonium chloride (2.14 g), sodium acetate trihydrate (5.44 g), water (10 ml), and ethanol (5 ml) were boiled together under reflux overnight. Ethyl acetate and aqueous sodium hydrogen carbonate were added; the aqueous layer was washed with ethyl acetate, freed from dissolved organic solvent at low pressure, cooled in ice, and acidified to pH 2 with dilute hydrochloric acid. The orange precipitate after being collected, washed, dried, and recrystallized twice (charcoal) from methanol-water afforded *dimethylisamic acid* (IV) as hydrated orange prisms (1.07 g); m.p. 121–124° (decomp.);  $\lambda_{\text{max}}$  412 ( $\epsilon$  1 500), 237 (34 750), and 206 nm (36 100);  $\nu_{\text{max}}$  3 470, 1 720, 1 612, and 1 230  $\text{cm}^{-1}$  (Found: C, 63.75; H, 4.95; N, 12.25.  $\text{C}_{18}\text{H}_{16}\text{N}_3\text{O}_3 \cdot \text{H}_2\text{O}$  requires C, 63.7; H, 5.05; N, 12.4%). The *methyl ester*, prepared with diazomethane, crystallized from methanol in orange prisms; m.p. 138–140°;  $\lambda_{\text{max}}$  416 ( $\epsilon$  1 420), 237 (30 300), and 207 nm (29 100);  $\nu_{\text{max}}$  1 730, 1 635, 1 617, and 1 220  $\text{cm}^{-1}$  (no peak between 3 600 and 3 100  $\text{cm}^{-1}$ ) (Found: C, 68.2; H, 5.1; N, 12.5.  $\text{C}_{19}\text{H}_{17}\text{N}_3\text{O}_3$  requires C, 68.05; H, 5.1; N, 12.55%). Both acid and ester dissolved in strong mineral acid to give violet solutions.

Dimethylisamic acid (247 mg) was boiled under reflux for 1 h with 0.1N-hydrochloric acid (50 ml). The cooled solution was extracted with ethyl acetate (5  $\times$  20 ml). The extracts on evaporation left 1-methylisatin (236 mg; 100.6% of theoretical), identified by m.p. (132–134°), mixed m.p., and i.r. spectrum. The aqueous acid layer was

freed from dissolved organic solvent, made alkaline, and distilled into standard hydrochloric acid; on titration, 0.72 mequiv. of volatile base (99% of theoretical) was found and the base was identified as ammonia.

*Resolution of Isamic Acid*.—Isamic acid (6.22 g) was suspended in propan-2-ol and added to a solution of brucine (from 10.13 g of brucine sulphate by shaking with ammonia and chloroform) in propan-2-ol, a total of 135 ml of the alcohol being used. The yellow slurry was heated to boiling and water (37 ml) was added gradually. From the resulting solution three crops of crystals (total 11.6 g) were obtained. On recrystallization of this material from 80% propan-2-ol it was noted that yellow, well-formed, sparingly soluble prisms tended to separate first (or to remain undissolved on warming) and that the needle-like crystals which sometimes separated were always contaminated by prisms, even after recrystallization of mechanically separated needles. Eventually it was recognized that interconversion of diastereoisomers must be occurring on recrystallization and the strategy finally adopted was to heat mother liquors in 80% propan-2-ol on a steam-bath during the day and to collect the prisms that separated overnight. In this way, despite handling losses, a total of 9.35 g of prisms, m.p. 193–196° (decomp.), was obtained. These all showed  $[\alpha]_{\text{D}}^{20} -0.5^\circ$  ( $c$  1.43 in 80% methanol) and may be substantially pure *brucine (+)-isamate* (Found: C, 64.05; H, 5.8; N, 9.9.  $\text{C}_{23}\text{H}_{26}\text{N}_2\text{O}_4 \cdot \text{C}_{16}\text{H}_{11}\text{N}_3\text{O}_3 \cdot 3\text{H}_2\text{O}$  requires C, 64.0; H, 5.9; N, 9.55%).

The salt (3.15 g) was decomposed by prolonged shaking with chloroform and aqueous ammonia (1.7N; 50 ml) until the layers separated clean and clear. The aqueous layer was extracted several times with chloroform and the excess of ammonia was removed at low pressure with slight warming. A sample (10 ml) of this solution (60 ml) was reserved; the remainder was carefully acidified with hydrochloric acid (2N) to pH 2. The red crystalline precipitate was collected, dried (1.1 g), and recrystallized rapidly without heating by dissolving it in methanol, concentrating the filtered solution, and carefully adding water. The fine red needles (0.85 g) of *(+)-isamic acid*, m.p. 159–165° (decomp.) had an i.r. spectrum almost identical with that of  $(\pm)$ -isamic acid (Found: C, 61.9; H, 3.85; N, 13.65.  $\text{C}_{16}\text{H}_{11}\text{N}_3\text{O}_3 \cdot \text{H}_2\text{O}$  requires C, 61.75; H, 4.2; N, 13.5%). In methanol ( $c$  0.235) it showed  $[\alpha]_{\text{D}}^{20} +90^\circ$  when fresh; after 3 days at 20 °C the specific rotation had sunk to  $+68^\circ$  and it was  $+54^\circ$  after 6 days.

The aqueous solution of the ammonium salt initially showed  $[\alpha]_{\text{D}}^{20} -6.6^\circ$  ( $c$  2.23; reckoned on anhydrous ammonium isamate). An aliquot portion diluted with 4 volumes of methanol was dextrorotatory:  $[\alpha]_{\text{D}}^{20} +1.8^\circ$ . The rotatory power of the aqueous solution fell steadily at 20 °C. After 8 weeks, when the rotation was *ca.* 6% of the initial value, the solution was concentrated somewhat at low pressure and acidified. The red crystalline precipitate was identified as  $(\pm)$ -isamic acid by its i.r. spectrum and its negligible optical rotation. The ammonium salt in 80% methanol appeared to undergo racemization more slowly.

To test whether the violet cation from *(+)-isamic acid* retained chirality, *(+)-isamic acid* (20 mg in 2 ml of methanol) was added to concentrated hydrochloric acid (1.652 ml). After 30 s the deep violet solution was poured into ice-cold potassium hydroxide (20% in methanol) equivalent to the hydrochloric acid. A control solution was made by mixing the acid and the alkali before adding the *(+)-isamic acid*. The filtered solutions were examined:

the control showed  $\alpha_D +0.351^\circ$  and the test solution  $\alpha_D +0.285^\circ$ .

The c.d. spectrum of (+)-isamic acid in methanol was kindly measured by Dr. G. Ryback. The maxima, minima, and crossover points ( $\Delta\epsilon$  in parentheses) were at 431 (+0.65), 332 (0), 309 (-3.54), 286 (-0.19), 277 (-0.75), 271 (0), 251 (+2.89), 245 (0), 230 (-20.1), 219 (0), and 210 nm (+28.5).

*Methyl(+)-isamate* was made from the (+)-acid and diazomethane and recrystallized from methanol: orange prisms; m.p. 192–193°;  $[\alpha]_D^{20} +88^\circ$  (*c* 0.25 in methanol). The *N*<sup>1</sup>-acetyl derivative of this ester formed orange prisms from ethyl acetate; m.p. 215–217°.

*Basic Strengths of Methyl 1-Acetyl-1,2-dihydro-2-oxo-spiro[3H-indole-3,2'(1H)-quinazoline]-4'-carboxylate* (VIII) and *Dimethyl 2-(2-Acetamidophenyl)-1,2-dihydroquinazoline-2,4-dicarboxylate* (IX).—The two substances (VIII) and (IX) were prepared as previously described.<sup>7</sup> It was noted that methanolysis of (VIII) was rapid even at room temperature and the u.v. spectra were taken in tetrahydrofuran [(VIII):  $\lambda_{\max}$  416 ( $\epsilon$  1 100), 236 (31 500), and 211 nm (28 200); (IX):  $\lambda_{\max}$  396 ( $\epsilon$  1 590), 240 (32 500), and 211 nm (29 400)]. In 8*N*-hydrochloric acid both substances formed violet solutions [(VIII):  $\lambda_{\max}$  526 ( $\epsilon$  1 310), 300 (4 890), 227sh (19 200), and 211 nm (21 600); (IX):  $\lambda_{\max}$  492 ( $\epsilon$  2 000), 320 (6 790), 228 (21 700), and 209 nm (24 300)]. The spectra in 8*N*- and in 6*N*-acid were identical. For measurement of basic strength a tetrahydrofuran solution (0.1 ml) of each substance (0.5 mg) was added to hydrochloric acid (5 ml) of normality 6, 4, 3, 2, and 1. The substance (VIII) separated at once from *N*-hydrochloric acid but fast scans could be taken with all other solutions [(VIII) separated also from 2*N*-hydrochloric acid after a time]. Absorbances at 530 nm [for (VIII)] and 490 nm [for (IX)] were measured, and *pK'* was calculated:<sup>9</sup> *pK'* for (VIII) was -0.46 and for (IX) it was +0.03. The acidic solutions were unstable, presumably because of hydrolysis to isatin.

*Methyl 6,12-Dihydro-12-hydroxy-6-oxoindolo[2,1-b]quinazoline-12-carboxylate* (*Methylisatoid*).—This was conveniently made by Hantzsch's<sup>13</sup> method: powdered *O*-methylisatin was dissolved in aqueous 90% acetic acid (4 ml per g). The crude product, which separated quickly, could be crystallized from pyridine-acetonitrile or, best, from aqueous dimethyl sulphoxide. It formed small compact orange prisms, m.p. 252°,  $\lambda_{\max}$  450 ( $\epsilon$  5 360), 319 (6 100), 272sh (7 100), 256 (17 500), 232 (13 400), and 201 nm (21 000);  $\nu_{\max}$  3 190br, 1 763, 1 730, 1 650, 1 610, and 1 595  $\text{cm}^{-1}$ .

*6,12-Dihydro-12-hydroxy-6-oxoindolo[2,1-b]quinazoline-12-carboxylic Acid*.—(a) Potassium isatin<sup>16</sup> (2.05 g) was dissolved in dimethylformamide (5 ml) and *O*-methylisatin (1.61 g) was added. The mixture was warmed gently to complete dissolution and acetic acid (0.6 ml) was added. After 3 days at room temperature the mixture was acidified with 2*N*-hydrochloric acid (5 ml) and the crystalline product (2.82 g) was collected. It was recrystallized, wastefully, by dissolution in cold ethanol, filtration, and addition of an equal volume of cold water. The *carboxylic acid* (XXVII) then formed orange rosettes, m.p. 231–235° (decomp.) (Found: C, 61.7; H, 3.7; N, 9.3.  $\text{C}_{16}\text{H}_{10}\text{N}_2\text{O}_4 \cdot \text{H}_2\text{O}$  requires C, 61.55; H, 3.85; N, 8.95);  $\lambda_{\max}$  450 ( $\epsilon$

5 480), 318 (6 720), 272sh (7 260), 255 (19 500), 232 (15 600), and 201 nm (24 000);  $\nu_{\max}$  3 550, 3 470, 1 740, 1 710, 1 660, 1 610, and 1 595  $\text{cm}^{-1}$  (characteristic broad  $\text{CO}_2\text{H}$  absorption between 3 300 and 2 400  $\text{cm}^{-1}$ ).

(b) *Methylisatoid* (0.5 g), sodium hydrogen carbonate (1 g), water (10 ml), and methanol (15 ml) were boiled together under reflux for 2.25 h. Methanol was removed at low pressure; the aqueous solution was cooled in ice and the product carefully precipitated with hydrochloric acid. The crystalline product (0.42 g) was recrystallized as in (a). The u.v. and i.r. spectra were identical with those of the product from method (a).

*12-Hydroxyindolo[2,1-b]quinazoline-6(12H)-one* (XXVIII).—The carboxylic acid (2.82 g) prepared by method (a) was dissolved in dimethyl sulphoxide and a little water was added. On warming to 60 °C a crystalline precipitate appeared rapidly. It was collected (2.45 g) and recrystallized from dimethyl sulphoxide, forming orange plates, m.p. 238–241° (lit.<sup>13</sup> 216–217°) (Found: C, 72.05; H, 4.05; N, 11.1. Calc. for  $\text{C}_{15}\text{H}_{10}\text{N}_2\text{O}_2$ : C, 72.0; H, 4.05; N, 11.2%);  $\lambda_{\max}$  450 ( $\epsilon$  5 375), 320 (6 750), 270 (14 400), 260 and 253 (16 300), 228 (13 600), and 201 nm (20 900);  $\nu_{\max}$  3 080, 2 740, 1 726, 1 655, 1 615, 1 603, and 1 575  $\text{cm}^{-1}$ . The same product was obtained from carboxylic acid prepared by method (b). In another experiment the carboxylic acid (50 mg) dissolved in acetone (10 ml) was heated under reflux for  $\frac{1}{2}$  h in an apparatus connected to a barium hydroxide trap. The mixture was cooled and  $\text{CO}_2$ -free air was led through the assembly; copious precipitation of barium carbonate was observed. The crystalline product that had separated from the acetone was identical with the product obtained as above (m.p. and i.r. spectrum). The substance was virtually insoluble in cold 2*N*-sodium hydroxide.

*Synthesis of Methylisatoid*.—The carboxylic acid (XXVII) (123 mg) was converted *via* the ammonium salt into the silver salt in the normal manner. The red salt was collected by centrifugation and washed by resuspension in water, aqueous ethanol, ethanol, and ether. It was then dried *in vacuo* in the dark, covered with methyl iodide (3 ml), and heated under reflux for 1 $\frac{1}{2}$  h. The excess of methyl iodide was evaporated off and the residue was extracted with hot acetonitrile, from which the crude product separated on addition of water. It was purified by trituration with cold 2*N*-sodium hydroxide, filtration, and immediate saturation with carbon dioxide. The orange precipitate after recrystallization from aqueous dimethyl sulphoxide was identical with methylisatoid (m.p., mixed m.p., and i.r. spectrum).

Reaction of the carboxylic acid with diazomethane gave a small yield of methylisatoid after extraction of the crude product with alkali as above. The major product was alkali-insoluble.

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<sup>16</sup> Gj. Stefanovic, Lj. Lorenc, R. I. Mamuzić, and M. Lj. Mihailović, *Tetrahedron*, 1959, **6**, 304.