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# Studies in the Biochemistry of Micro-organisms

## 99. METABOLIC PRODUCTS OF *ASPERGILLUS VERSICOLOR* (VUILLEMIN) TIRABOSCHI*

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The first examination in this Laboratory of the metabolic products obtained from the mycelium of *Aspergillus versicolor* (Vuillemin) Tiraboschi was undertaken by Abou-Zeid (1953). He isolated in very small yield two new coloured crystalline metabolites, *A*, m.p. 240–241°, and *B*, m.p. 233–234°, in addition to mannitol. The amounts of *A* and *B* obtained were insufficient for complete characterization but a few properties and colour reactions were noted.

One of us (Hammady, 1954), using a later isolation of the same organism, obtained the same three products. Product *A*, formed in relatively good yield, was obtained as pale-yellow needles of m.p. 247°, and was allocated the molecular formula $C_{18}H_{12}O_6$. The general properties, derivatives and some breakdown products were described.

Davies, Roberts & Wallwork (1956) recently announced that early in 1955 they had commenced an investigation of *A. versicolor* and had obtained from the dried mycelium of one strain a metabolite crystallizing in pale-yellow needles of m.p. 243°. They drew attention to the work of Hatsuda & Kuyama (1954) and of Hatsuda, Kuyama & Terashima (1954), who had isolated the same metabolite and named it sterigmatocystin. Hatsuda *et al.* (1954) ascribed to sterigmatocystin the molecular formula $C_{15}H_{12}O_5$ and allocated to it a structural formula. Davies *et al.* (1956), adopting the name sterigmatocystin, determined the molecular weight by the Rast method and by the X-ray crystallographic method. From these results and the combustion figures they concluded that the molecular formula for sterigmatocystin is $C_{18}H_{14}O_6$, both empirical and structural formulae given by Hatsuda *et al.* (1954) being thus incorrect.

It was then evident that product *A* isolated in this Laboratory was the same as sterigmatocystin. Through the courtesy of Dr Roberts we were able to compare our product directly with a specimen isolated by the Nottingham School; a mixed-melting-point determination showed they were identical. Since the work carried out on this subject in this School has been reported only in Ph.D. theses of London University it is appropriate to record briefly for wider circulation the results therein embodied which relate to the metabolic products of *A. versicolor*.

Some strains of the fungus when grown on Czapek–Dox medium produced a coloured mycelium, which, after drying, was extracted with solvents. Light petroleum afforded an orange-coloured solid from which product *A* (sterigmatocystin) was obtained. On subsequent ether extraction of the mycelium product *B* was isolated. It formed orange needles of m.p. 233–234°. Further extraction of the mycelium with methanol afforded mannitol.

Sterigmatocystin is optically active and contains one methoxyl group. The following derivatives have been prepared: diacetyl, monomethoxy, monoethoxy compounds and a brominated derivative. When refluxed with 25% ethanolic potassium hydroxide, sterigmatocystin furnished an optically inactive isomer which afforded a monomethyl ether and a diacetate. This product, which we term *iso*-sterigmatocystin, shows an ultraviolet-absorption curve very similar to that of sterigmatocystin, indicating that the conjugated system giving rise to the absorption is still present. Some change other than mere racemization must have occurred,

* Part 98: Neelakantan, Pocker & Raistrick (1956).

however, since *iso*sterigmatocystin is readily soluble in aqueous sodium hydroxide whereas sterigmatocystin is insoluble in this reagent. On distillation of sterigmatocystin with zinc dust a small amount of crystalline material was isolated, which from its ultraviolet-absorption spectrum appeared to be anthracene or a homologue thereof. On heating sterigmatocystin with fused potassium hydroxide resorcinol was obtained together with acetic acid. The combustion figures for the diacetate of sterigmatocystin indicate that it has the empirical formula $C_{22}H_{18}O_9$, and thus contains a molecule of water in excess of the formula for a simple diacetate; this may, of course, be water of crystallization. *iso*Sterigmatocystin forms the normal diacetate $C_{22}H_{16}O_8$. No evidence of the presence of a reactive carbonyl group in sterigmatocystin could be obtained. The figures obtained for methoxyl determinations on sterigmatocystin and its derivatives were consistently too high; this appears to be a characteristic of the substance.

Sterigmatocystin contains six oxygen atoms. One of these is present as a methoxyl group, two further oxygen atoms are present as hydroxyl groups, one of which can be methylated and both of which can be acetylated. This leaves a further three oxygens whose function is not as yet ascertained. Little can be said of the carbon skeleton in the light of this work, although the production of resorcinol by potassium hydroxide fusion and of anthracene by zinc-dust distillation suggest the presence of one or more aromatic rings. There may be present an aliphatic link containing the asymmetric carbon atom which confers optical activity. The hydroxyl group which resists methylation but not acetylation could well be part of this structure. Further work to elucidate the structure is by mutual agreement to be undertaken by the Nottingham School, who inform us that they are now in accord with our molecular formula of $C_{18}H_{12}O_6$ for sterigmatocystin (personal communication from Dr J. C. Roberts).

For product *B*, the evidence obtained is too scanty to permit any definite conclusions as to its nature. Whereas the colour reactions and the ultraviolet absorption agree well with a polyhydroxyanthraquinone structure, the hydrogen:carbon ratio as determined by combustion is too high to accord with this hypothesis. Product *B* does not appear to be identical with versicolorin (cf. Hatsuda & Kuyama, 1955; Hatsuda, Kuyama & Terashima, 1955) since it differs considerably in melting point and also shows optical activity.

## EXPERIMENTAL

All melting points are uncorrected. Elementary analyses and acetyl determinations are by Drs Weiler and Strauss, Oxford, except where otherwise stated. Methoxyl determinations are by one of us (I.M.M.H.).

### *Organism*

The culture originally used in this investigation (Abou-Zeid, 1953) was a strain (L.S.H.T.M. Cat. no. 247) of *Aspergillus versicolor* (Vuillemin) Tiraboschi received from the Timber Research Laboratories, South Africa (their Cat. no. 2543). This afforded only minimal yields of sterigmatocystin, but slightly larger amounts of product *B*. In the continuation of this work (Hammady, 1954) a strain (L.S.H.T.M. Cat. no. A 262) was employed which was isolated in this Laboratory from an Australian-soil sample by Mrs S. Marcus. When grown on Czapek–Dox glucose medium the fungus produced a mycelium which was convoluted and yellowish white on the upper surface with brown droplets, the reverse being brown to orange–brown in colour. On extraction it afforded sterigmatocystin in resonable yield, product *B* in minimal yield.

During the course of the investigation thirteen other strains of the organism were examined for production of the characteristic products. Of these, eight produced no pigment, four were inefficient pigment producers, but one was found to afford sterigmatocystin in better yield than strain A 262, although the yield of *B* was again minimal. With this strain (L.S.H.T.M. Cat. no. Ac 59), obtained from the Centraalbureau voor Schimmelcultures Baarn (ex Bainier), the mycelium after incubation for 21 days was orange to green in colour on the upper surface, with orange reverse. In the later work this strain Ac 59 was used in place of A 262 to prepare a stock of sterigmatocystin.

### *Method of culture*

The usual Czapek–Dox medium of the following composition was employed: $NaNO_3$, 2·0 g.; $KH_2PO_4$, 1·0 g.; KCl, 0·5 g.; $MgSO_4,7H_2O$, 0·5 g.; $FeSO_4,7H_2O$, 0·01 g.; glucose, 50 g.; water to 1 l. The medium was distributed (350 ml./flask) in 1 l. conical flasks plugged with cotton wool and sterilized by steaming on three successive days. Flasks were inoculated with a spore suspension from wort–agar slope cultures and incubated at 24° in the dark, usually for 21 days.

### *Harvesting and extraction of mycelium*

The mycelium was separated from the culture solution by filtration, washed with water, pressed and dried in a vacuum at about 40°. It was then ground in a coffee mill and exhaustively extracted with light petroleum (b.p. 40–60°). The orange-coloured solid which separated consisted of crude sterigmatocystin (product *A*). It was repeatedly crystallized from amyl acetate, from which it separated as long pale-yellow needles, m.p. 247–248°. The yield was 40–50% of the crude material.

The mycelium was then extracted with ether. From the extract after concentration a dark brownish orange solid separated. This was crude product *B*. After several recrystallizations from $CHCl_3$ it was obtained as orange needles, m.p. 231–234°. The yield of pure material was only about 2·5% of the crude product.

Further extraction of the mycelium with methanol afforded brownish crystals, which by recrystallization were obtained as colourless needles, m.p. 166°. This product was identified as D-mannitol. The data obtained for four batches of strain A 262 and six batches of strain Ac 59 are shown in Table 1.

Table 1. *Culture characteristics and yields of crude products of strain A* 262 (*batches* 1–4) *and strain Ac* 59 (*batches* 5–10) *of* Aspergillus versicolor (*Vuillemin*) *Tiraboschi when grown on Czapek–Dox glucose medium at* 24°

| Batch no. | No. of flasks | Period (days) | Residual glucose (%) | Wt. of dry mycelium (g.) | Crude sterigmatocystin Wt. (g.) | Crude sterigmatocystin M.p. | Crude product *B* Wt. (g.) | Crude product *B* M.p. |
|---|---|---|---|---|---|---|---|---|
| 1 | 99 | 21 | 0·48 | 454 | 5·4 | 198–228° | 2·6 | 180–200° |
| 2 | 100 | 21 | 0·98 | 400 | 4·7 | 210–220 | 4·2 | 180–220 |
| 3 | 100 | 21 | 0·52 | 490 | 10·4 | 190–220 | 6·0 | 170–190 |
| 4 | 99 | 21 | 0·37 | 492 | 5·2 | 210–225 | 2·8 | 160–190 |
| 5 | 100 | 21 | 0·47 | 390 | 18·0 | 212–227 | 2·9 | 160–200 |
| 6 | 100 | 24 | 0·64 | 375 | 15·0 | 210–220 | 2·0 | 190–220 |
| 7 | 100 | 21 | 0·74 | 390 | 16·1 | 210–220 | 1·9 | 190–220 |
| 8 | 100 | 21 | 0·70 | 390 | 22·5 | 210–222 | 3·3 | 140–180 |
| 9 | 148 | 28 | 0·29 | 605 | 19·5 | 198–212 | 2·3 | 190–210 |
| 10 | 100 | 21 | 0·79 | 390 | 19·4 | 208–228 | 1·6 | 200–220 |

### *Properties of sterigmatocystin*

When crystallized from amyl acetate sterigmatocystin forms long, pale-yellow needles of m.p. 247–248°. When the pure product was recrystallized from ethanol the m.p. dropped to 242–243°. The pure product sublimes unchanged *in vacuo* and then has m.p. 247°; $[\alpha]^{20}_{5461}$ −485°, $[\alpha]^{20}_{5790}$ −420°, $[\alpha]^{20}_{D}$ −398° in $CHCl_3$ (*c*, 1); light-absorption max., 250 and 326 mμ. in $CHCl_3$ (log ε 4·82 and 4·48 respectively). [Found: C, 65·8, 66·2, 66·7, 66·4; H, 4·0, 3·9, 4·0, 3·8; *C*-Me, nil; OMe (mean of several determinations) 10·8 %. Mol.wt. (Rast) 344. Determination by Schoeller on a sublimed sample: C, 66·55, 66·5; H, 3·8, 3·8. $C_{18}H_{12}O_6$ requires C, 66·7; H, 3·7; one OMe group 9·6 %. Mol.wt. 324.] It is sparingly soluble in most organic solvents in the cold, but is readily soluble in cold $CHCl_3$. It is insoluble in water and in aqueous NaOH. Its ethanolic solution gives a stable pale-green colour with ethanolic $FeCl_3$. It gives a green solution in conc. $H_2SO_4$ with very weak yellow fluorescence.

### *Derivatives of sterigmatocystin*

*Diacetyl sterigmatocystin.* Sterigmatocystin (0·5 g.), anhydrous sodium acetate (1 g.) and acetic anhydride (5 ml.) were heated together in an oil bath at 140–150° for 6 hr. The mixture after cooling was poured into water with stirring and the solid obtained (0·4 g.) was collected and crystallized from pentanol or methanol giving the *diacetate* as colourless rods, m.p. 228–229°, $[\alpha]^{22}_{5461}$ −237° in ethanol (*c*, 3·8), which gave no colour with ethanolic $FeCl_3$. (Found: C, 61·9; 61·8; H, 4·3, 4·35; $CH_3CO$, 24·6, 24·8; OMe, 7·7. $C_{22}H_{16}O_8$,$H_2O$ requires C, 61·95; H, 4·25; two $CH_3CO$ groups, 20·2; one OMe group, 7·3 %.)

*Monomethyl ether of sterigmatocystin.* Sterigmatocystin (0·5 g.) in dry acetone (50 ml.) was refluxed for 18 hr. with anhydrous $K_2CO_3$ (4 g.), and methyl iodide (7 g.) was added gradually at intervals. The acetone was distilled off and the residue treated with water. The insoluble material was crystallized from methanol, which afforded colourless needles of the *monomethyl ether*, 0·26 g., m.p. 271–272°; $[\alpha]^{22}_{5461}$ −459° in ethanol (*c*, 1). These gave no colour with ethanolic $FeCl_3$, and in conc. $H_2SO_4$ an emerald-green colour. (Found: C, 67·45; H, 4·5; OMe, 19·2, 19·9. $C_{19}H_{14}O_6$ requires C, 67·45; H, 4·2; two OMe groups, 18·3 %.)

*Monoethyl ether of sterigmatocystin.* Sterigmatocystin (0·54 g.) in dry acetone (200 ml.) was refluxed with anhydrous $K_2CO_3$ (10 g.), and ethyl iodide (15 ml.) was added gradually during 18 hr. The product was treated with water after removal of acetone. The insoluble solid (0·48 g.) was crystallized from methanol, then from ethanol. The *monoethyl ether* separated as colourless needles, 0·2 g., m.p. 261–262°; $[\alpha]^{22}_{5461}$ −443° in ethanol (*c*, 1). (Found: C, 67·9, 67·8; H, 4·65, 4·7. $C_{20}H_{16}O_6$ requires C, 68·2; H, 4·6 %.)

### *Bromination of sterigmatocystin*

On the addition of saturated bromine water (15 ml.) to a solution of sterigmatocystin (0·24 g.) in dioxan (15 ml.) an immediate yellow ppt. formed. This was collected, washed with water and dried. The *product* (0·42 g.) crystallized from ethanol in yellow needles, m.p. 181–182° (decomp.), light-absorption max., 258 and 331 mμ. in ethanol ($E^{1\%}_{1\,cm.}$ 619 and 257 respectively). It was sparingly soluble in conc. aqueous NaOH. [Found: C, 36·5, 35·9; H, 2·0, 2·4; Br, 43·7, 44·0; OMe, 5·0, 5·0. Determination by I.C.I. Ltd., Welwyn: C, 35·8, 35·9; H, 2·4, 2·4; Br, 42·4, 42·35. The best fit, $C_{17}H_{13}O_7Br_3$, requires C, 35·9; H, 2·3; Br, 42·1; one OMe group, 5·45 %. A more likely formula, $C_{18}H_{13}O_7Br_3$ (based on the addition of 4 Br and subsequent replacement of one Br by OH), requires C, 37·2; H, 2·3; Br, 41·3; OMe, 5·3 %.]

### *Distillation of sterigmatocystin with zinc dust*

An intimate mixture of sterigmatocystin (1 g.) and electrolytic-zinc dust (15 g.) was placed in a Pyrex tube (15 mm. diam., length of column 20 cm.) and sandwiched between two columns (10 cm. long) of zinc dust. The column was slowly heated (starting from the exit end of the tube) while a slow current of hydrogen was passed. A brown oil formed in the exit and gradually solidified. A fraction of this insoluble in light petroleum was sublimed (0·027 mg.). This had m.p. 195–196°. The u.v. absorption spectrum in ethanol closely resembled that of anthracene or β-methylanthracene but the $\lambda_{max.}$ agreed slightly better with those of anthracene. It is concluded that a product of zinc-dust distillation has the anthracene nucleus.

### *Fusion of sterigmatocystin with potassium hydroxide*

Sterigmatocystin (2 g.), KOH (20 g.) and water (5 ml.) were stirred and heated slowly in a nickel crucible to 300–320°, at which temperature the melt was maintained for 20 min. The product, after cooling, was dissolved in water and extracted with ether, acidified and again extracted. The second ether extract was a brown gum, which slowly

began to crystallize. On sublimation a small amount of crystalline material was obtained. By further sublimations and recrystallization of the product from xylene and benzene colourless needles were eventually obtained of m.p. 110·5–111° (yield 0·2 g.) which gave a violet colour with aqueous $FeCl_3$. This was shown by the u.v. absorption spectrum and by a mixed m.p. determination with authentic material to be resorcinol. (Found: C, 65·5; H, 5·2. Calc. for $C_6H_6O_2$: C, 65·45; H, 5·5%.) The dibenzoate, m.p. 117–118°, showed no depression in m.p. when mixed with an authentic sample of dibenzoylresorcinol of the same m.p., prepared for comparison.

In a repetition of the KOH fusion the volatile acids liberated by acidification were distilled *in vacuo*. The neutralized distillate, concentrated to 3 ml., was treated with *p*-bromophenacyl bromide (1·2 g., the amount calculated from the titration) in 50% (v/v) aqueous ethanol (10 ml.) and the solution was refluxed for 2 hr. A crystalline product (1·06 g.) which separated after recrystallization from light petroleum formed colourless rods, m.p. 84–85°. The mixed m.p. with an authentic sample of *p*-bromophenacyl acetate, m.p. 84°, showed no depression.

### *Action of ethanolic potassium hydroxide on sterigmatocystin*

Sterigmatocystin (0·35 g.) was refluxed with 25% ethanolic KOH (20 ml.). The substance dissolved completely in 6 hr. The heating was continued for a further 26 hr., and the solution became dark brown. After concentration at reduced pressure it deposited a light-brown solid which dissolved in water. Acidification to Congo red afforded a ppt. (0·2 g.) which after four crystallizations from *n*-butanol formed pale yellowish brown plates of *iso*sterigmatocystin, m.p. 234°; light-absorption max., 252 and 336 mμ. in ethanol (log ε 4·62 and 4·23 respectively). (Found: C, 66·7; H, 3·9; OMe, 11·1, 11·3. $C_{18}H_{12}O_6$ requires C, 66·7; H, 3·7; one OMe group, 9·6%.) Admixture with sterigmatocystin depressed the m.p. slightly (to 231°). The product was optically inactive and was readily soluble in 2N-NaOH, the solution being coloured yellow. It gave a green colour with ethanolic $FeCl_3$.

*Diacetate of* iso*sterigmatocystin*. This was prepared in similar manner to the diacetate of sterigmatocystin. From ethanol the *diacetate* was obtained in pale-brown needles, m.p. 197–198°. (Found: C, 64·25; H, 3·9; $CH_3.CO$, 22·2. $C_{22}H_{16}O_8$ requires C, 64·7; H, 3·9; two $CH_3.CO$ groups, 21·1%.)

*Methyl ether of* iso*sterigmatocystin*. This was prepared in the same way as the corresponding product of sterigmatocystin and crystallized from a mixture of $CHCl_3$ and light petroleum; the *methyl ether* was obtained as colourless rods, m.p. 162–163°. (Found: C, 67·2; H, 4·6; OMe, 20·5. $C_{19}H_{14}O_6$ requires C, 67·45; H, 4·2; two OMe groups, 18·3%.)

### *Product* B (*Abou-Zeid*, 1953)

The dry mycelium (400–500 g.) obtained from cultures of a strain of *A. versicolor* (L.S.H.T.M. Cat. no. 247) grown on Czapek–Dox medium was exhaustively extracted with light petroleum and then with ether. The solid separating from the ether was purified by crystallization from $CHCl_3$ and *product* B was thus obtained as long orange needles, m.p. 233–234°, unchanged after further recrystallization; $[\alpha]_{5790}^{22}$ – 178° in ethanol (*c*, 0·37); light-absorption max. 228, 266, 294, 324 and 453 mμ. in ethanol (log ε, assuming mol.wt. 316 as required by $C_{17}H_{18}O_6$, 4·15, 4·14, 4·37, 3·90 and 3·91 respectively). The yield was only 0·8–1·6 g. from 100 flasks. It contained no nitrogen, halogen, sulphur or methoxyl. (Found: C, 63·9, 64·0; H, 5·7, 5·6. $C_{17}H_{18}O_6$ requires C, 64·1; H, 5·7%.) It is insoluble in water but soluble in cold ethanol, hot $CHCl_3$ and hot benzene. It gives a red colour with ethanolic $FeCl_3$, carmine with cold conc. $H_2SO_4$ and purple-red with 2N-NaOH. It dissolves in aqueous $Na_2CO_3$ to give a pink-violet solution and is slightly soluble in aqueous bicarbonate giving a pink solution.

Crystalline compounds obtained in attempts to methylate or acetylate the product were isolated only in minute amounts insufficient for analysis.

### *Identification of mannitol*

By extraction of the mycelium of strains L.S.H.T.M. Cat. no. 247 and A 262 with methanol after exhaustive extraction with (*a*) light petroleum and (*b*) ether, a brown solid was obtained from each strain. This product, on recrystallization from methanol, separated as colourless needles, m.p. 166°. Its m.p. was undepressed on admixture with an authentic sample of D-mannitol. A portion of the product was converted into the hexa-acetate of m.p. 126°. There was no depression in m.p. on admixture with an authentic sample of D-mannitol hexa-acetate, m.p. 126°.

## SUMMARY

1. Sterigmatocystin, a crystalline metabolic product obtained from the mycelium of *Aspergillus versicolor* (Vuillemin) Tiraboschi by extraction with light petroleum was isolated as pale-yellow needles of m.p. 247°, $[\alpha]_D^{20} - 398°$. It contains one methoxyl group and possesses the molecular formula $C_{18}H_{12}O_6$.

2. The following derivatives of sterigmatocystin were prepared; diacetate (hydrate), m.p. 228°, monomethyl ether, m.p. 271°, monoethyl ether, m.p. 261°.

3. On fusion with potassium hydroxide sterigmatocystin yields resorcinol and acetic acid, and on zinc-dust distillation small amounts of anthracene or a homologue.

4. When heated with 25% ethanolic potassium hydroxide sterigmatocystin furnishes an optically inactive isomer, *iso*sterigmatocystin, m.p. 234°, yielding a diacetate, m.p. 197°, and a methyl ether, m.p. 162°.

5. A second optically active crystalline product (*B*), m.p. 233°, was obtained in small yield from the mycelium by ether extraction.

6. Mannitol was extracted by methanol from the mycelium as a third metabolic product.

The ultraviolet-absorption spectra were determined with a Uvispek spectrophotometer purchased by means of a grant from the Central Research Fund of London University. We thank Imperial Chemical Industries Ltd., The Frythe, Welwyn, for carrying out the elementary analysis of the bromo compound from sterigmatocystin.

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# Studies in the Biochemistry of Micro-organisms

## 100. METABOLITES OF *PENICILLIUM ATROVENETUM* G. SMITH. PART I. ATROVENETIN, A NEW CRYSTALLINE COLOURING MATTER*

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*Penicillium atrovenetum* G. Smith is a new species which was described recently by Mr George Smith of this Department (1956). Its specific name was given because colonies of the mould on Czapek agar are at first bright bluish green (*venetus*) in colour but rapidly turn greyer and become almost black (*ater*) in age. The reverse of the colonies is a deep yellowish brown. This species is closely related morphologically to *P. herquei* Bainier & Sartory and should therefore be placed in the *P. herquei* series of the Biverticillata-Symmetrica as described by Raper & Thom (1949). Smith (1956) proposes, however, that a better placement of the *P. herquei* series is in the Asymmetrica-Velutina in close association with the *P. citrinum* and *P. brevi-compactum* series.

During the routine chemical examination of a large number of species of moulds it was found here that the mycelium of the type strain and also of two different strains of *P. atrovenetum*, grown on Czapek–Dox glucose–mineral salts solution, produced some interesting reactions. Immersion of portions of the pressed mycelium in ethanol yielded an orange-brown extract which gave an intensely dark-olive brown, almost black ferric reaction. Other portions immersed in cold concentrated sulphuric acid gave an immediate yellow to orange-brown solution with a strong green fluorescence. We have isolated the fungal colouring matter which is responsible for these colour reactions and, since it has not been described previously, we propose for it the name atrovenetin.

For the bulk preparation of atrovenetin *P. atrovenetum* strain S.M. 683 was cultivated on Czapek–Dox glucose solution for 21 days at 24° in the dark. The mould mycelium was then separated, washed with water, dried *in vacuo* and ground to a fine powder. The powder was extracted with light petroleum to remove 'fat' and then with ether. From the ether extracts, initially very dark green in colour, crude atrovenetin separated as a dark powder from which much of the dark material was separated by extraction with acetone. The residual yellow-brown colouring matter was purified by crystallization from dioxan or acetone. The yield of crystalline atrovenetin obtained averaged about 23 g. from 100 flasks containing 35 l. of Czapek–Dox solution and constituted about 43 % of the total ether extract. The combined weights of the light petroleum and ether extracts amounted to 18–20 % of the dry weight of the mould mycelium.

Atrovenetin, $C_{19}H_{18}O_6$, forms yellow-brown plates or prisms, which melt with decomposition at 295° and sublime in high vacuum at about 210°. Solutions of atrovenetin in dioxan are strongly dextrorotatory. The molecule of atrovenetin contains a minimum of four hydroxyl groups, no methoxyl group and at least three or probably four methyl groups attached to carbon. It is not readily soluble in the usual organic solvents. It is not soluble in sodium carbonate or sodium bicarbonate solution but dissolves readily in aqueous sodium hydroxide to an orange solution. Its solution in cold concentrated sulphuric acid is bright yellow in colour with a characteristic intense yellow-green fluorescence. It also gives a characteristic intense red-brown ferric reaction in ethanol solution.

The following functional derivatives of atrovenetin have been prepared: atrovenetin hydrochloride, $C_{19}H_{18}O_6.HCl$, yellow needles, m.p. 285–286° (decomp.); atrovenetin perchlorate,

$$C_{19}H_{18}O_6.HClO_4,2H_2O,$$

* Part 99: Birkinshaw & Hammady (1957).