898. *The Chemistry of Fungi. Part XXXIX.*[1] *The Structure of Monascin.*

By B. C. Fielding, J. S. E. Holker, D. F. Jones, A. D. G. Powell, K. W. Richmond, Alexander Robertson, and W. B. Whalley.

Structure (III), suggested for monascin, is shown to be compatible with the physical and chemical properties of the parent pigment and its derived compounds. Evidence has been obtained that the parent pigment may contain small amounts of the bis-homologue (V). The nuclear magnetic resonance spectra of monascin and its derivatives are discussed in an Appendix.

In 1895 it was shown [2] that the fungus *Monascus purpureus* Went. was the organism responsible for the colour of " Red Rice " ("Ang Khak "), a native preparation used as a colouring matter for foodstuffs and alcoholic beverages in parts of Java and China, and which was made by allowing the fungus to grow on the surface of boiled rice.[3] In 1932 Karrer and Saloman [4] described the isolation of a yellow pigment, monascin, from " Red Rice," and at about the same time Nishikawa [5] reported the isolation of a red compound, monascorubrin, together with a yellow compound, monascoflavin (apparently identical with monascin) from a culture of *M. purpureus* grown on a synthetic medium. More recently a systematic survey in this Department of the metabolites of the *Monascus* genus [6] has resulted in the isolation of monascin and rubropunctatin from *M. rubropunctatus* Sâto, monascin and " monascorubrin " from *M. purpureus* Went., and monascin from *M. rubiginosus* Sâto. We have described the structural elucidation of rubropunctatin (I),[1,1a] and have made a preliminary report [7] on the nature of" monascorubrin " in which it was shown by mass-spectrometric examination of suitable derivatives that this substance is a mixture of minor amounts of rubropunctatin (I) with a higher homologue, monascorubrin. Since oxidation of the monascorubrin–rubropunctatin mixture gave octanoic acid

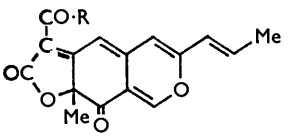
[1] Part XXXVIII, Haws and Holker, *J.*, 1961, 3820.
[1a] Haws, Holker, Kelly, Powell, and Robertson, *J.*, 1959, 3598.
[2] Went, *Ann. d. Sci. Nat. Bot.*, 1895, [8], **1**, 1.
[3] Vorderman, *Analecta ob Cromatologisch Gebied.* II. *Geneesh. Fydschrift voor Ned. Indie*, 1894, **34**, No. 5.
[4] Karrer and Saloman, *Helv. Chim. Acta*, 1932, **15**, 18.
[5] Nishikawa, *J. Agric. Chem. Soc. Japan*, 1932, **8**, 1007.
[6] Powell, Robertson, and Whalley, *Chem. Soc. Special Publ.*, No. 5, 1957, p. 27.
[7] Fielding, Haws, Holker, Powell, Robertson, Stanway, and Whalley, *Tetrahedron Letters*, 1960, No. 5, 24.

and smaller amounts of hexanoic acid, whereas under similar conditions rubropunctatin gave only hexanoic acid, it has been suggested that monascorubrin has structure (II), differing from rubropunctatin (I) only in the length of the saturated side-chain. The present paper is concerned with the third of these metabolites, monascin.

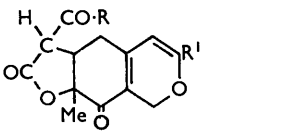
In preliminary examinations of monascin Karrer and his co-workers [4,8] showed that it was an optically active yellow pigment, which seemed to have the molecular formula, $C_{20}H_{24}O_5$. The compound was devoid of methoxyl groups and did not give a ferric reaction. Although it contained one active hydrogen atom (Zerewitinoff), and was precipitated by carbon dioxide from solution in sodium hydroxide, monascin was recovered unchanged after attempted acetylation and benzoylation. Oxidation formed hexanoic, acetic, and oxalic acid, and ozonolysis produced acetaldehyde and methylglyoxal. Hydrogenation of monascin in the presence of a platinum catalyst, and termination of the reaction after absorption of one molecular equivalent of hydrogen, gave small amounts of a compound, m. p. 130—131°, which was formulated as a dihydro-derivative. Exhaustive hydrogenation of monascin in acetic acid with a platinum catalyst resulted in the uptake of four molecular equivalents of hydrogen to give an oily perhydro-derivative. A microcrystalline dihydro-derivative was isolated by reduction of monascin with zinc and acetic acid.

It was apparent that the formula, $C_{20}H_{24}O_5$, suggested for monascin, could not be regarded as rigidly established and, indeed, our analyses on monascin and its derivatives were more compatible with the formula $C_{21}H_{26}O_5$ for the parent pigment. To differentiate between these formulæ tetrahydromonascin (see below) was analysed in the mass spectrometer when, despite extensive fragmentation, the peak due to the largest ion was at *m/e* 362, corresponding to the formula $C_{21}H_{30}O_5$ for tetrahydromonascin, and hence to $C_{21}H_{26}O_5$ for monascin.

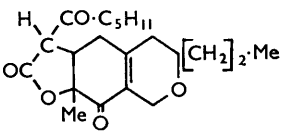
It was tempting to suggest at an early stage that monascin might be a tetrahydro-derivative of rubropunctatin for the following reasons: (*a*) monascin and rubropunctatin (I) are co-metabolites of *M. rubropunctatus*, (*b*) both give hexanoic acid and acetaldehyde, on oxidation and ozonolysis respectively, and (*c*) the molecular formulæ differ only by four hydrogen atoms. The evidence obtained in the present investigation strongly supports this hypothesis and suggests that monascin has structure (III). For convenience the reactions to be discussed will be interpreted in terms of this structure.



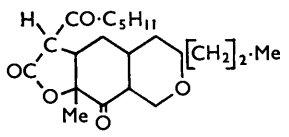
(I); R = C_5H_{11}
(II); R = C_7H_{15}



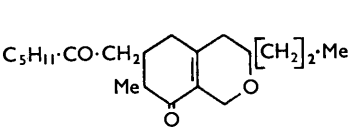
(III); R = C_5H_{11}, R' = CH:CHMe
(IV); R = C_5H_{11}, R' = $[CH_2]_2$·Me
(V); R = C_7H_{15}, R' = CH:CHMe



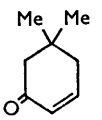
(VI)



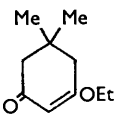
(VII)



(VIII)



(IX)



(X)

Dihydro- (IV), tetrahydro- (VI), and hexahydro- (VII) derivatives of monascin have been prepared by carefully controlled hydrogenation. In this connection the product, m. p. 130—131°, reported as a dihydro-derivative by Karrer and Saloman [4] appears to be the tetrahydro- (VI) (m. p. 135°) rather than the dihydro-derivative (IV) (m. p. 119—121°). Further hydrogenation of hexahydromonascin occurred readily to give an oil. However,

[8] Geiger and Karrer, *Helv. Chim. Acta*, 1941, **24**, 289.

since the ultraviolet absorption spectrum of hexahydromonascin shows no significant end-absorption, it seems likely that, in agreement with structure (VII), this compound contains no olefinic bonds. Hence, the further hydrogenation of hexahydromonascin must involve reduction of carbonyl functions. In agreement with this view, the infrared spectrum of the hexahydro-derivative shows strong absorption in the carbonyl region but no bands which could be attributed either to a hydroxyl group or a double bond, whereas the oily perhydro-substance shows hydroxyl absorption and a significant decrease in the intensity of carbonyl absorption.

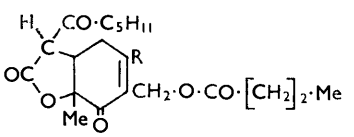
In agreement with structures (III) and (IV) for monascin and its dihydro-derivative, respectively, ozonolysis of (III) gave acetaldehyde whereas, under comparable conditions, (IV) gave no volatile aldehydic or ketonic fragment. The reported [8] isolation of methylglyoxal from ozonolysis of monascin in carbon tetrachloride has been reinvestigated. Thus, in an attempted isolation of methylglyoxal as its 2,4-dinitrophenylosazone, a small amount of a sticky red solid (3% of the calculated amount for the presence of one $>C{:}CH{\cdot}CMe{:}C<$ group in the formula $C_{21}H_{26}O_5$) was obtained; it had an infrared spectrum similar to that of authentic methylglyoxal 2,4-dinitrophenylosazone. In view of the very low yield of this product, it seems likely that the compound is an artefact and has no direct structural implication.

The suggested structures for monascin and its hydro-derivatives are compatible with the ultraviolet absorption spectra. Thus, in agreement with the presence of a fully substituted αβ-unsaturated ketone system in structure (VI) for tetrahydromonascin, the spectrum has $\lambda_{max.}$ 244 and 320 mμ (log ε 4·01 and 1·93), and the nuclear magnetic resonance spectrum shows no peak which could be attributed to vinyl hydrogen. Empirical calculation of the expected $\lambda_{max.}$ for the linear conjugated system in structure (IV) for dihydromonascin gives: 215 (parent enone)[9] + 30 (double-bond extending conjugation)[9] + 10 (α-substituent)[9] + 12 (β-substituent)[9] + 18 (γ-substituent)[9] + 39 (homoannular diene component)[9] + 50 (auxochromic ethereal oxygen)[10] = 374 mμ. Since the principal high-intensity absorption band occurs at $\lambda_{max.}$ 365 mμ (log ε 4·18) the agreement with the calculated figure is reasonable, particularly when it is realised that the bathochromic effect of 50 mμ due to the ethereal oxygen atom is assessed by comparison with a limited number of compounds in which the analogy is not particularly close.[10] Finally, the hypsochromic shift of 25 mμ observed on conversion of monascin into its dihydro-derivative is lower than the more usual figure of 30 mμ associated with the removal of a conjugated double bond from a linear conjugated unsaturated ketone. This may be due to the fact that whereas the π-*p*-conjugation of the auxochromic oxygen is fully exerted in the linear conjugated system of dihydromonascin, the same conjugation in monascin is not fully exerted owing to the crossed π–π-conjugation of the propenyl double bond.

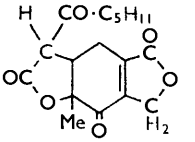
Reduction of tetrahydromonascin with zinc and acetic acid at room temperature gave tetrahydroapomonascin (VIII), a small amount of hexahydromonascin (VII), and carbon dioxide in an amount proportional to the amount of compound (VIII) formed. This reductive expulsion of carbon dioxide from tetrahydromonascin is reminiscent of the behaviour of rubropunctatin derivatives under similar conditions [1a] and is associated with the presence of the structural unit $R{\cdot}CO{\cdot}\overset{|}{C}{\cdot}CO{\cdot}O{\cdot}\overset{|}{C}{\cdot}CO{\cdot}R'$. Unlike monascin and its hydrogenated derivatives, tetrahydroapomonascin is devoid of active hydrogen (Zerewitinoff) and is insoluble in dilute sodium hydroxide. Hence the acidity of compounds (III), (IV), (VI), and (VII) must be associated with the tertiary hydrogen attached to the α-carbon of the β-ketolactone system. Reduction of monascin under similar conditions also gave carbon dioxide, but in this case no other recognisible product could be isolated. In direct contrast, hexahydromonascin was unchanged after treatment with zinc and acetic acid at room temperature and it appears, therefore, that the double bond of the

[9] Fieser and Fieser, "Steroids," Reinhold Publishing Corporation, New York, 1959, pp. 18—21.
[10] Bowden, Braude, and Jones, *J.*, 1946, 948.

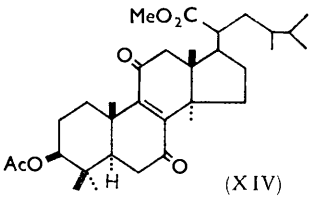
αβ-unsaturated ketone group in monascin and its tetrahydro-derivative is necessary for reduction under these very mild conditions. This is in agreement with similar findings in the steroid series where reduction of α-ketols and their acetates with bivalent metals is greatly facilitated by the presence of double bonds, halogen atoms, or additional acetoxyl groups attached to the carbon atom alpha to the ketol structure.[11]



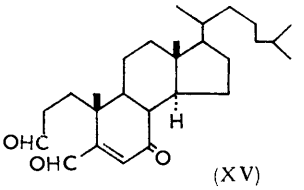
(XI); R = CHO (XII); R = CO_2H



(XIII)

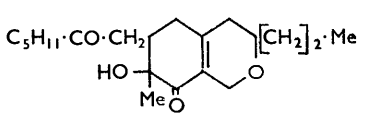


(XIV)

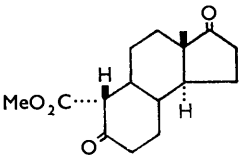


(XV)

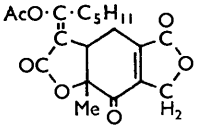
The infrared spectra of monascin and its derivatives support the suggested structures. Thus, the spectrum of tetrahydroapomonascin (VIII) has bands at 1709 (side-chain ketonic carbonyl), 1656, and 1629sh cm.$^{-1}$ (αβ-unsaturated ketone). Hexahydromonascin (VII) has bands at 1786 (γ-lactone), 1739 (cyclohexanone carbonyl), 1718 cm.$^{-1}$ (side-chain ketonic carbonyl). The high wave-numbers of the γ-lactone and cyclohexanone bands in this spectrum are in keeping with their relationship in an α-ketol lactone system, since it is known that the ester and ketonic carbonyl bands of α-ketol esters are similarly displaced.[12] Corresponding shifts are also apparent in the spectrum of tetrahydromonascin (VI) which has bands at 1786 (displaced γ-lactone), 1718 (side-chain ketonic carbonyl), 1695 (displaced carbonyl stretch of αβ-unsaturated ketone), and 1623 cm.$^{-1}$ (double-bond stretch of αβ-unsaturated ketone). The carbonyl stretching regions in the spectra of monascin (III) and its dihydro-derivative (IV) are very similar, both having bands at 1785 (displaced γ-lactone), 1715 (side-chain ketonic carbonyl), 1669, and 1628 cm.$^{-1}$ (αβ-unsaturated ketone). The band at 1669 cm.$^{-1}$ in these compounds is at a lower wave number than the corresponding band (1695 cm.$^{-1}$) in the spectrum of the tetrahydro-derivative (VI). This is due to the fact that the trienone and dienone systems of monascin and its dihydro-derivative form part of a vinylogous ester system. Thus we find an equivalent shift between the vinylogous ester system of dimedone ethyl ether (X) (1634 cm.$^{-1}$) and the αβ-unsaturated ketone system of 5,5-dimethylcyclohex-2-en-1-one (IX) (1670 cm.$^{-1}$).



(XVI)



(XVII)



(XVIII)

Direct chemical evidence has been obtained for the presence of a vinyl ether system in dihydromonascin (IV). Thus, oxidation with sodium dichromate in aqueous acetic

[11] Rosenfeld, *J. Amer. Chem. Soc.*, 1957, **79**, 5540.

[12] Bellamy and Williams, *J.*, 1957, 861; Dickson and Page, *J.*, 1955, 447; Jones and Herling, *J. Org. Chem.*, 1954, **19**, 1252.

acid gave a mixture of the formyl-butyrate ester (XI) and the corresponding carboxylic acid (XII). The former compound was converted into the latter by further oxidation under similar conditions. Hydrolysis of the acid-ester (XII) gave n-butyric acid and the dilactone (XIII). The formyl-ester (XI) similarly gave butyric acid on hydrolysis but in this case the second product was a gum. The dilactone (XIII) has also been prepared directly from both monascin and its dihydro-derivative by oxidation with chromic oxide in sulphuric acid, in conditions where tetrahydromonascin is relatively stable.

The presence of an aldehyde group in compound (XI) and a carboxyl group in (XII) has been demonstrated. Thus, the former compound reduced Tollens's reagent and readily gave a crystalline mono-2,4-dinitrophenylhydrazone whereas, under similar conditions the acid (XII) and the dilactone (XIII) reacted very slowly to give amorphous products. The acid (XII) reacted rapidly with sodium hydrogen carbonate, and with diazomethane gave a neutral amorphous substance which seems to be essentially the methyl ester, since it generated the dilactone (XIII) on hydrolysis. The ultraviolet absorption spectra of the aldehyde (XI) and the acid (XII) had $\lambda_{max.}$ 238, 324 mμ (log ε 3·95, 2·24) and $\lambda_{max.}$ 242 mμ (log ε 3·85), respectively. The *K*-band due to the enedione chromophore in the aldehyde (XI) is at a very low wavelength compared with the band at 270 mμ owing to the similar chromophore in methyl 3-acetoxy-7,11-dioxoeburic-8-en-21-oate (XIV).[13] However, in the latter compound both carbonyl groups of the chromophore are present as keto-groups rigidly locked in a cyclic system, whereas in the former, one of the carbonyl groups is in an aldehyde function. A better analogy to the aldehyde (XI) is provided by compound (XV), which has the *K*-band at 242 mμ [14] in close agreement with the spectrum of (XI).

In accordance with the presence of an αβ-unsaturated lactone ring in the dilactone (XIII), this compound, unlike monascin, gave a positive Legal test under the modified conditions of Paist, Blout, Uhle, and Elderfield.[15] Furthermore, the dilactone reduced Tollens's reagent. This is in agreement with the αβ-unsaturated γ-lactone system in structure (XIII) being unsubstituted at the γ-position, since compounds containing this structural unit are known to isomerise under the alkaline conditions of this test to give the anions of the corresponding γ-formyl-carboxylic acids.[15] The acid (XII) also reduced Tollens's reagent, again owing to the formation of an aldehyde. Zerewitinoff estimation on the dilactone showed two active hydrogen atoms compared with one in dihydromonascin. Since β-cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide and other αβ-unsaturated γ-lactones give values of from half to one active hydrogen atom in this estimation,[15] the value for compound (XIII) is reasonable. The ultraviolet absorption spectrum of this compound provides further evidence for the structural features present. Thus, although the spectrum has bands at 214 and 241 mμ, typical of αβ-unsaturated lactone and αβ-unsaturated ketone functions, respectively, it is apparent that these functions must be present in a cross-conjugated system since the molar extinction coefficients (7100 and 5000, respectively) are only about half the values associated with the separate functions, *i.e.*, β-cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide and tetrahydromonascin have $\lambda_{max.}$ 212 (ε 15,000) and 244 mμ (ε 10,000), respectively.

The evidence already presented defines all the features of structure (III) for monascin apart from the angular methyl group. The location of this is based primarily on deductions from nuclear magnetic resonance spectra which, incidentally, provide strong confirmatory evidence for the complete structure (see Appendix). However, some chemical evidence has been obtained for the position of this methyl group. Thus, treatment of tetrahydromonascin with barium hydroxide and acidification of the reaction mixture gave a non-acidic gum, which showed bands at 3448, 1709, and 1667 cm.$^{-1}$ in its infrared spectrum. The absence of absorption at 1770 cm.$^{-1}$ suggested that this product was essentially the α-ketol (XVI) arising from tetrahydromonascin by hydrolysis of the γ-lactone function with subsequent

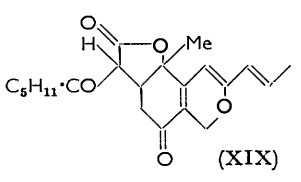
[13] Holker, Powell, Robertson, Simes, and Wright, *J.*, 1953, 2414.
[14] Personal communication from Professor B. Lythgoe, F.R.S.
[15] Paist, Blout, Uhle, and Elderfield, *J. Org. Chem.*, 1941, **6**, 273.

decarboxylation of the resultant β-keto-acid. In keeping with structure (XVI), the product reacted with lead tetra-acetate to give an acidic gum, which, unlike tetrahydromonascin, gave a positive iodoform reaction. Hence this acidic oxidation product apparently contained a methyl ketone function, in agreement with the position of the angular methyl group in the monascin structure.

Since the suggested structures for monascin and its hydrogenated derivatives contain a β-keto-lactone system having one hydrogen on the α-carbon atom, the absence of enolic properties in these compounds must be considered. In this connection it is known that no enolic properties are shown by compounds containing β-keto-ester systems in which the generation of an enol is sterically or energetically unfavourable, *e.g.*, compound (XVII).[16] Since there do not appear to be any adverse steric factors in structure (III) for monascin, it seems likely that energetic factors operate, *e.g.*, if the γ-lactone ring in this structure is *trans*-fused, the generation of an enol would be energetically unfavourable. There is some evidence that tetrahydromonascin (VI) does form an enol acetate. Thus, treatment of this compound with acetic anhydride and pyridine gave an amorphous product which regenerated compound (VI) on hydrolysis with dilute sodium hydroxide. The infrared spectrum of the amorphous product, which showed no absorption attributable to a saturated ketone function, is in agreement with the enol acetate structure. Acetylation of the dilactone (XIII) gave a crystalline monoacetate which was reconverted into the parent by hydrolysis. This acetate is tentatively formulated as (XVIII) despite the presence in its infrared spectrum of a band at 1724 cm.$^{-1}$ which is best attributed to a saturated carbonyl function. This band may well be due to the cyclic ketone group in structure (XVIII), since it is known from ultraviolet data that the corresponding group in the dilactone (XIII) is not fully conjugated with the αβ-double bond.

Finally, in view of the co-occurrence of monascin and " monascorubrin " in *M. purpureus*, and since the latter substance is known to be a mixture of rubropunctatin (I) and the higher homologue monascorubrin (II), it seemed likely that monascin from this source might also be a similar mixture of compounds (III) and (V). Comparative mass spectrometric examination of tetrahydromonascin samples derived from *M. purpureus* and *rubropunctatus* has shown no molecular ions of *m/e* greater than 362 [corresponding to structure (III)] but, in view of the extensive fragmentation under the experimental conditions, the possibility cannot be discounted that small amounts (<15%) of a homologue of type (V) are present in one or both samples. To test this hypothesis further, samples of monascin derived from both fungi have been oxidised with potassium permanganate and the steam-volatile acids products isolated. Paper chromatographic examination has revealed in each case a major spot due to hexanoic acid (which has been independently characterised as the *p*-bromophenacyl ester [8]) and a trace of a minor spot of higher R_F value which may be due to octanoic acid arising from a compound of structure type (V). However, since a compound of this type must be present in very minor amounts, and in any case would be difficult to separate, it has been ignored in the present studies.

[*Added in proof.* After submission of this paper structure (XIX) was proposed [20] for monascin (monascoflavin). In the absence of experimental detail in the Japanese paper we are unable to assess the evidence for this structure. However, in our opinion the production of iodoform by successive reaction of tetrahydromonascin with barium hydroxide, lead tetra-acetate, and iodine in sodium hydroxide is difficult to reconcile with structure (XIX) for monascin. Further, the formation of tetrahydroapomonascin from tetrahydromonascin under very mild reductive conditions, together with the infrared spectral data for hexahydromonascin, are not readily explicable in terms of structure (XIX).]



(XIX)

[16] Sondheimer, Mechoulam, and Sprecher, *Tetrahedron Letters*, 1960, No. 22, 38.

Appendix

Nuclear Magnetic Resonance Spectra of Monascin and its Derivatives.

By L. M. Jackman, Chemistry Department, Imperial College of Science and Technology

The spectra of monascin (III), dihydro- (IV), tetrahydro- (VI), and tetrahydroapomonascin (VIII) were determined at 56·4 Mc./sec. for solutions in deuterochloroform. The spectrum of monascin exhibits a complex band between 3·0 (τ) and 4·0 which undoubtedly arises from the olefinic protons of the disubstituted double bond. These protons, together with the three protons of the methyl group on the double bond, constitute an ABX_3 nuclear spin system. A detailed analysis was not attempted since it could be shown by a double irradiation technique * that a strong doublet at 8·14 (olefinic methyl) corresponded to the X_3 absorption of the ABX_3 system. By using the same technique it was possible to simplify the complex AB region to an isolated AB pattern from which the coupling constant ($J = 15$ c./sec.) between the olefinic protons could be determined. The magnitude of this coupling constant establishes a *trans*-configuration for the double bond. All lines of the ABX_3 system were absent from the spectra of the above derivatives of monascin. The olefinic region of the spectrum of monascin also contained a singlet at 4·40. This band was found at a higher frequency (4·76) in dihydromonascin but was absent from the spectra of compounds (VI) and (VIII). The diamagnetic shift of this absorption, which accompanies hydrogenation of the disubstituted double bond shows that the di- and tri-substituted double bonds are directly conjugated,[17] thus establishing the partial structure $\cdot CH{=}\overset{|}{C}\cdot CH{=}CH\cdot CH_3$.

The spectra of both monascin and dihydromonascin exhibit an AB system near 5·2 ($\tau_A = 4{\cdot}97$, $\tau_B = 5{\cdot}27$, $J = 12{\cdot}9$ c./sec., and $\tau_A = 5{\cdot}01$, $\tau_B = 5{\cdot}31$, $J = 12{\cdot}9$ c./sec., respectively). The positions of these bands are consistent with absorption arising from a methylene group which is both allylic and oxygenated,[17] and the coupling constant has the value expected for two protons attached to the same sp^3-hydridised carbon atom. The same band is present in the spectra of compounds (VI) and (VIII) but in both cases it is displaced by 0·40 p.p.m. to higher frequencies. This diamagnetic shift is expected, since hydrogenation of the central double bond of the triene system increases the electron density at the ether oxygen atom. The widths of the lines of the AB system suggest that the methylene protons are weakly coupled with other allylic protons.

A very sharp doublet is observed near 6·09 in the spectra of compounds (III), (IV), and (VI), but not in (VIII). This band must therefore arise from the single proton of the β-keto-lactone system. The coupling constant ($J = 12{\cdot}5$ c./sec.) is large and suggests that this proton is coplanar with and probably *cis* to that at the angular position between the lactone and the carbocyclic ring.[17] Another feature common to the spectra of (III), (IV), and (VI), but absent from that of (VIII) is a strong, sharp singlet at 8·50 which must arise from a methyl group at a fully substituted carbon atom. Its position is that expected for a methyl group attached to the same carbon atom as a carbonyl group and an oxygen atom.[17]

The fact that all the well-defined features of the complex spectra of monascin and its derivatives can be accommodated by structure (III) provides strong supporting evidence for it.

* A refined technique for the double irradiation of protons has been developed by Dr. D. W. Turner and details of the method will shortly be published.

[17] Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon, London, 1959.

Experimental

Unless otherwise stated, ultraviolet absorption spectra were measured for 95% alcohol solutions with a Unicam S.P. 500 spectrophotometer, infrared spectra for chloroform solutions with a Perkin-Elmer model 21 instrument, and optical rotations for chloroform solutions at room temperature (17—21°).

Monascin (III) *and "Monascorubrin" from* Monascus purpureus *Went.*—A culture of this organism, obtained from the Centraal-bureau voor Schimmelcultures, Baarn, Holland, was grown on the medium described by Nishikawa [5] at 30° for 21—28 days until pigmentation was complete. Maceration of the undried red mycelial mats with cold ether was continued until the extract was no longer highly coloured. The crude pigment, isolated by concentration of the extract, was resolved by fractional crystallisation from ether into "monascorubrin," red needles (from ether), m. p. 134—136° (decomp.), "monascorubramine," purple needles (from benzene), m. p. 198° (decomp.), and monascin, yellow needles (from ether or alcohol), m. p. 142—144° (decomp.), $[\alpha]_D$ +544° (*c* 1·27), $\lambda_{max.}$ 231, 287, and 390 mμ (log ε 4·18, 3·60, and 4·21) (Found: C, 70·7; H, 7·5; *C*-Me, 10·5; active H, 0·25; OMe, 0. Calc. for $C_{21}H_{26}O_5$: C, 70·4; H, 7·3; 3*C*-Me, 12·2; 1 active H, 0·28%). In an alternative procedure for the separation of monascin, the pigment mixture (3 g.) in ether (1 l.) was shaken with aqueous ammonia (*d* 0·88) (18 ml.) in water (750 ml.). After addition of concentrated hydrochloric acid (25 ml.), the ether layer was separated, dried (Na_2SO_4), and evaporated. The residue in benzene (250 ml.) was chromatographed on a column (12 in. × 1 in.) of silica gel. Elution with benzene–ether (9 : 1) gave monascin (1·8 g.), and benzene–acetone (1 : 1) gave "monascorubramine" (0·5 g.). When separated in this way, the pigment obtained from 500 penicillin flasks, each containing 250 ml. of culture medium, gave approximately 40 g. of monascin and 10 g. of "monascorubramine."

Further concentration of the ether extract from the mycelium gave a white solid which was resolved by fractional crystallisation from ether into ergosterol, needles (from methanol), m. p. and mixed m. p. 161° (*O*-acetate, m. p. and mixed m. p. 171°), and stearic acid, needles (from light petroleum), m. p. and mixed m. p. 68° (amide, m. p. and mixed m. p. 106°).

Monascin and Rubropunctatin (I) *from* M. rubropunctatus *Satô.*—When grown on Czapek–Dox solution [18] as previously described,[1a] this organism gave monascin, m. p. 142—144° (decomp.) (Found: C, 70·6; H, 7·5%), and rubropunctatin, m. p. 156—157° (decomp.) (Found: C, 71·2; H, 6·3. Calc. for $C_{21}H_{22}O_5$: C, 71·2; H, 6·3%).

Monascin from M. rubiginosus *Satô.*—This organism, obtained from the Centraal-bureau voor Schimmelcultures, Baarn, Holland, was grown at 30° for 28 days in 390 penicillin flasks, each containing Nishikawa's solution [5] (500 ml.). The dried, milled mycelium (1000 g.) was percolated with light petroleum (b. p. 60—80°) and the extract concentrated (to 500 ml.). Monascin, which separated on cooling, was crystallised from ether, giving yellow needles (4·3 g.), m. p. and mixed m. p. 142—143° (decomp.). Further concentration (to 300 ml.) of the light-petroleum extract gave ergosterol, plates (from methanol), m. p. and mixed m. p. 158—161°, $[\alpha]_D$ −127° (*c* 1·13) [*O*-acetate, plates (from methanol–ether), m. p. and mixed m. p. 173—174°].

Monascin was extracted from ether with 2N-sodium hydroxide and was reprecipitated from the alkaline solution with carbon dioxide. The pigment gave a positive hydroxamic acid test, a negative iodoform reaction, developed no colour in the Legal test,[15] and did not reduce Fehling's or Tollens's reagent.

Dihydromonascin (IV).—Hydrogenation of monascin (1·0 g.) in alcohol (100 ml.) at atmospheric pressure with 5% palladium–barium sulphate (500 mg.) was complete in 1 hr., and 1 mol. of gas had then been absorbed. On isolation, *dihydromonascin* separated from aqueous alcohol in pale yellow needles (950 mg.), m. p. 119—121° (decomp.), $[\alpha]_D$ +445° (*c* 1·01), $\lambda_{max.}$ 248 and 365 mμ (log ε 3·60 and 4·18) (Found: C, 70·2; H, 8·0; active H, 0·25. $C_{21}H_{28}O_5$ requires C, 70·0; H, 7·8; 1 active H, 0·28%).

Tetrahydromonascin (VI).—Hydrogenation of monascin (1·0 g.) in alcohol (200 ml.) at atmospheric pressure in the presence of 10% palladium–carbon (500 mg.) was terminated after 2·2 mol. of gas had been absorbed (*ca.* 1 hr.). The filtered solution was concentrated (to 15 ml.) and diluted with a few drops of water. On cooling, a colourless solid separated; it was recrystallised from aqueous alcohol giving *tetrahydromonascin* (500—650 mg.) as needles, m. p.

[18] Raistrick and Rintoul, *Phil. Trans.*, 1931, *B*, **220**, 2.

135°, $[\alpha]_D$ +245° (*c* 1·02) [Found: C, 69·5; H, 8·2; active H, 0·29%; *M* (mass spectrometry), 362. $C_{21}H_{30}O_5$ requires C, 69·6; H, 8·3; 1 active H, 0·27%; *M*, 362]. The same compound (500—650 mg.) was also prepared from dihydromonascin (1·0 g.) by a similar procedure, the reaction being terminated after absorption of 1·1 mol. of gas.

Acetylation of tetrahydromonascin (200 mg.) with acetic anhydride (3 ml.) and pyridine (1 ml.) at room temperature for 15 hr., and isolation of the product in ether in the usual way, gave a gum (189 mg.), $\nu_{max.}$ 1770s and 1689s cm.$^{-1}$. On treatment with 2N-sodium hydroxide (15 ml.) for 15 min., this material (180 mg.) in methanol (5 ml.) regenerated tetrahydromonascin (130 mg.), needles (from aqueous alcohol), m. p. and mixed m. p. 135°.

Hexahydromonascin (VII).—Hydrogenation of tetrahydromonascin (200 mg.) in alcohol (30 ml.) at atmospheric pressure in the presence of 10% palladium–carbon (100 mg.) was terminated after 1·1 mol. of gas had been absorbed. Concentration of the filtered solution gave *hexahydromonascin* as fine needles (60 mg.), m. p. 208—209°, $[\alpha]_D$ +75° (*c* 1·00) (Found: C, 69·0; H, 8·7; active H, 0·28. $C_{21}H_{32}O_5$ requires C, 69·2; H, 8·8; 1 active H, 0·27%). This experiment was difficult to reproduce, the reaction time and yield varying with each batch of catalyst.

Hexahydromonascin, in ethanol, slowly absorbed hydrogen (*ca.* 1 mol.) at atmospheric pressure in the presence of 10% palladium–carbon to give a glass, $\nu_{max.}$ 3500m, 1786s, and 1718m cm.$^{-1}$.

Ozonolysis of Monascin and its Dihydro-derivative.—(*a*) *In ethyl acetate.* A stream of ozone and oxygen was led into a solution of monascin (400 mg.) in ethyl acetate (30 ml.) at −70° until absorption was complete (*ca.* 2 hr.). The pale yellow solution was evaporated *in vacuo*, and the residue treated with water (15 ml.) for 15 hr. at room temperature. After removal of the insoluble material, acetaldehyde was isolated from the aqueous solution in steam and characterised as the 2,4-dinitrophenylhydrazone (120 mg., 48%), yellow blades (from alcohol), m. p. and mixed m. p. 164—166°. Ozonolysis of dihydromonascin (500 mg.) under similar conditions gave no volatile aldehydic or ketonic product.

(*b*) *In carbon tetrachloride.* Monascin (400 mg.), in carbon tetrachloride (150 mg.), was treated with ozone and oxygen at room temperature until the yellow colour of the solution was discharged (4 hr.). After removal of the solvent under reduced pressure and decomposition of the residual ozonide with zinc dust (1 g.) and water (25 ml.) at room temperature for 12 hr., acetaldehyde was isolated from the reaction mixture by distillation in methanol, and characterised as the 2,4-dinitrophenylhydrazone (90 mg., 36%; m. p. 165—166°). The aqueous phase (after distillation with methanol) was distilled in steam into a solution of 2,4-dinitrophenylhydrazine hydrochloride. The resultant deep orange precipitate (20 mg.) was adsorbed on neutral alumina from solution in benzene. Chloroform–benzene (1 : 1) eluted a sticky red solid (15 mg.), m. p. 280—290°, which could not be purified further. The infrared spectrum (in mineral oil dispersion) was very similar to that of authentic pyruvaldehyde 2,4-dinitrophenylosazone, m. p. 300° (decomp.). Ozonolysis of dihydromonascin under parallel conditions gave no volatile aldehydic or ketonic product.

Tetrahydroapomonascin (VIII).—Zinc dust (8 g.) was added in one portion to a vigorously stirred solution of tetrahydromonascin (1 g.) and anhydrous sodium acetate (2 g.) in acetic acid (50 ml.) at room temperature. After 1 hr. more zinc dust (2 g.) was added and stirring continued for a further 1 hr. Carbon dioxide evolved during the reaction was estimated as barium carbonate (Found: 240 mg. of $BaCO_3$. 1 CO_2 corresponding to 400 mg. of tetrahydroapomonascin requires 300 mg. of $BaCO_3$). After filtration, the original reaction mixture was concentrated (to 10 ml.), diluted with water (50 ml.), and extracted with ether (3 × 80 ml.). The ether extract was washed in turn with 2N-sodium hydroxide (5 × 50 ml.) and water (3 × 50 ml.), dried (Na_2SO_4), and evaporated. The residual gum was crystallised from light petroleum (b. p. 60—80°) giving *tetrahydroapomonascin* as long needles (400 mg.), m. p. 102°, $[\alpha]_D$ +175° (*c* 1·00), $\lambda_{max.}$ 243 mμ (log ε 4·03) (Found: C, 75·1; H, 10·2; *C*-Me, 11·8. $C_{20}H_{32}O_3$ requires C, 75·0; H, 10·0; 3*C*-Me, 14·1%). Acidification (HCl) of the 2N-sodium hydroxide washings gave a buff-coloured solid which separated from methanol as fine needles (30 mg.) of hexahydromonascin, m. p. and mixed m. p. 208—209°.

Reduction of monascin (500 mg.) in ethanol (10 ml.) and acetic acid (25 ml.) with zinc dust (5 g.) under similar conditions gave carbon dioxide (Found: 80 mg. of $BaCO_3$; 1 CO_2 from 500 mg. of monascin requires 276 mg. of $BaCO_3$). Partition of the residue in the usual way gave "neutral" (200 mg.) and "acidic" (200 mg.) fractions which could not be crystallised.

Under similar conditions hexahydromonascin (100 mg.) gave only unchanged starting material (92 mg.), m. p. and mixed m. p. 208—209°, and no carbon dioxide could be detected.

Oxidation of Dihydromonascin with Sodium Dichromate.—Solutions of dihydromonascin (1 g.) in acetic acid (40 ml.) and sodium dichromate (2·5 g.) in water (10 ml.) were mixed and kept at room temperature for 15 hr. The reaction mixture was diluted with water (50 ml.) and poured into an excess of 2N-sodium hydrogen carbonate (*ca.* 1 l.). Isolated in ether and crystallised from benzene, the *formyl-butyrate* (XI) formed small needles (400 mg.), m. p. 183° (decomp.), $[\alpha]_D$ +212° (*c* 1·00); $\nu_{max.}$ 1789s (γ-lactone), 1742sh ($CO_2C_3H_7$), 1718s (saturated ketone), and 1684s cm.$^{-1}$ (O=C·C=C·CHO) (Found: C, 64·4; H, 7·5; *C*-Me, 7·90. $C_{21}H_{28}O_7$ requires C, 64·2; H, 7·2; 3*C*-Me, 11·5%). The sodium hydrogen carbonate solution was acidified with concentrated hydrochloric acid and extracted with ether (5 × 50 ml.), and the extract evaporated. On treatment of the residue with water (50 ml.) a precipitate was obtained which was crystallised from ice-cold ether giving the *monohydrate* of the acid ester (XII) as micro-needles (200 mg.), m. p. 143° (decomp.), $[\alpha]_D$ + 144° (*c* 1·00); $\lambda_{max.}$ 242 mμ (log ε 3·85); $\nu_{max.}$ (mineral oil dispersion) 3367(m + b) (hydrate), 2618(w + b) (·CO_2H), 1786s (γ-lactone), 1736sh (·$CO_2C_3H_7$), 1718s (saturated ketone), 1701sh (·CO_2H) and 1689sh cm.$^{-1}$ (sh) (O=C·C=C·CO_2H) (Found: C, 59·1; H, 7·1; *C*-Me, 9·7. $C_{21}H_{28}O_8,H_2O$ requires C, 59·0; H, 7·0; 3*C*-Me, 10·5%). Evaporation of this hydrate with benzene gave the *anhydrous acid* (Found: C, 61·0; H, 6·9. $C_{21}H_{28}O_8$ requires C, 61·8; H, 6·9%).

Compounds (XI) and (XII) reduced Tollens's and Fehling's reagents. The aldehyde (XI) reacted immediately with an alcoholic solution of 2,4-dinitrophenylhydrazine sulphate to give a *mono-2,4-dinitrophenylhydrazone*, orange needles, m. p. 270° (decomp.) (Found: N, 9·4. $C_{27}H_{32}O_{10}N_4$ requires N, 9·8%). The acid (XII) reacted slowly with the same reagent giving an amorphous orange solid. Treatment of the acid (XII) with diazomethane in ether gave a glass; $\nu_{max.}$ 1786s, 1739s, 1724s, and 1709sh cm.$^{-1}$.

Oxidation of the aldehyde (XI) (500 mg.) with sodium dichromate (1·2 g.), as described for dihydromonascin, gave the monohydrate of the acid (XII) (200 mg.), m. p. and mixed m. p. 143° (decomp.), and unchanged aldehyde (XI) (150 mg.), m. p. and mixed m. p. 183° (decomp.).

The Dilactone (XIII).—(*a*) To monascin (1 g.) in acetone (40 ml.) at room temperature was added, dropwise, a solution (*ca.* 4 ml.) of chromium trioxide (13·5 g.) in sulphuric acid (11·5 ml.) and water (25 ml.) until the red-brown colour due to excess oxidising agent persisted for 10 min. The reaction mixture was then diluted with water (100 ml.) and extracted with ether (4 × 100 ml.) and the ether solution partitioned with saturated sodium hydrogen carbonate solution (5 × 80 ml.) and 2N-sodium hydroxide (5 × 40 ml.) into "neutral" (trace), "acidic" (850 mg. of intractable brown gum), and "enolic" (120 mg. of pale yellow solid) fractions. A solution of the "enolic" fraction in benzene (30 ml.) was chromatographed on silica gel (15 × 1 cm.). Chloroform–benzene (1 : 1) eluted the *dilactone* (XIII), which separated from benzene as small needles (100 mg.), m. p. 188°, $[\alpha]_D$ +222° (*c* 1·00), $\nu_{max.}$ 1789s (saturated γ-lactone), 1770s (αβ-unsaturated γ-lactone), 1721s (saturated ketone), and 1692m, sh cm.$^{-1}$ (αβ-unsaturated ketone) (Found: C, 63·7; H, 6·6; active H, 0·64; *C*-Me, 8·5. $C_{17}H_{20}O_6$ requires C, 63·7; H, 6·3; 2 active H, 0·63; 2*C*-Me, 9·3%).

(*b*) Oxidation of dihydromonascin (1 g.) by the above procedure gave an intractable "acid" fraction (850 mg.) and the dilactone (XIII), needles (85 mg.), m. p. and mixed m. p. 188°, from benzene. Under similar conditions, oxidation of tetrahydromonascin (500 mg.) gave an intractable acid fraction (40 mg.) and unchanged starting material, needles (420 mg.), m. p. and mixed m. p. 135°, from aqueous alcohol.

(*c*) The acid (XII) (500 mg.) was dissolved in 2N-sodium hydroxide (10 ml.) and, after 3 min. at room temperature, the solution was acidified with 2N-sulphuric acid. n-Butyric acid was separated in steam, isolated as its sodium salt, and converted, in the usual way, into the anilide which formed long needles (130 mg.), m. p. and mixed m. p. 95°, from light petroleum (b. p. 60—80°) (Found: C, 74·0; H, 8·4; N, 8·7. Calc. for $C_{10}H_{13}NO$: C, 73·6; H, 8·0; N, 8·6%). The dilactone (XIII), which was isolated in ether from the residue after steam distillation, separated from benzene in needles (320 mg.), m. p. and mixed m. p. 188°. Hydrolysis of the aldehyde (XI) (400 mg.), under similar conditions, gave n-butyric acid [characterised as the anilide (100 mg.), m. p. and mixed m. p. 95°] and an intractable brown gum (250 mg.).

(*d*) Treatment of the glassy methyl ester of acid (XII) (50 mg.) with methanol (0·2 ml.)

and 2N-sodium hydroxide (1 ml.) for 10 min. at room temperature, followed by acidification, gave the dilactone (XIII), small needles (23 mg.), m. p. and mixed m. p. 188°, from benzene.

The dilactone (XIII) reduced Fehling's and Tollens's reagents but did not give a ferric test or an iodoform reaction. Prepared with acetic anhydride and pyridine, the *monoacetate* (XVIII) separated from methanol in needles, m. p. 212°; $\lambda_{max.}$ 218 and *ca.* 240 infl. mμ (log ε 4·26 and 4·03); $\nu_{max.}$ 1779—1767(vs + b), 1727s, 1689m, and 1157s cm.$^{-1}$ (Found: C, 63·1; H, 6·4; active H, 0·26; *O*-Ac, 14·8. $C_{19}H_{22}O_7$ requires C, 63·0; H, 6·1; 1 active H, 0·27; 1 *O*-Ac, 12·0%). This compound (100 mg.), moistened with methanol (0·5 ml.), dissolved slowly in 2N-sodium hydroxide. Acidification gave the parent dilactone (80 mg.), m. p. and mixed m. p. 188°.

On reduction of the dilactone (XIII) with zinc and acetic acid at room temperature, carbon dioxide was produced in about the same molar proportion as from monascin under similar conditions.

Degradation of Tetrahydromonascin with Barium Hydroxide.—Tetrahydromonascin (500 mg.) in methanol (10 ml.) and a saturated solution of barium hydroxide in water (100 ml.) were heated together, for 1 hr., under reflux in an atmosphere of nitrogen. After acidification of the boiling solution with concentrated hydrochloric acid, the mixture was extracted with ether and the ether solution washed with 2N-sodium hydroxide (3 × 50 ml.) and water (50 ml.), and dried (Na_2SO_4). Evaporation of the ether left a colourless gum (380 mg.); $\nu_{max.}$ 3448m, 1709s, and 1667s cm.$^{-1}$. This material (100 mg.) in acetic acid (8 ml.) and water (2 ml.) was oxidised with finely powdered lead tetra-acetate (150 mg.) for 15 min. at room temperature. After dilution of the mixture with water (30 ml.), the product was separated in ether (3 × 20 ml.), extracted with saturated sodium hydrogen carbonate solution (3 × 20 ml.), and after acidification of this solution, re-extracted into ether (3 × 20 ml.). To a solution of this acidic material in 2N-sodium hydroxide (1 ml.) were added, dropwise, iodine (1 g.) and potassium iodide (2 g.) in water (4 ml.) until a brown colour due to excess of iodine persisted for 5 min. This colour was removed with a few drops of 2N-sodium hydroxide and the mixture diluted with water (50 ml.); iodoform (40 mg. 33% of calc. amount) separated, m. p. and mixed m. p. 118°.

Oxidation of Monascin and the Dilactone (XIII) *with Potassium Permanganate.*—Potassium permanganate (1 g.) in water (50 ml.) was added dropwise during ½ hr. to a boiling solution of monascin (200 mg.), moistened with methanol (0·5 ml.), in 2N-sodium hydroxide (25 ml.). The reaction mixture was acidified with dilute sulphuric acid, clarified with sulphur dioxide, and then distilled in a current of steam. The volatile acids were isolated in ether and a small amount of this solution was spotted on to a strip of filter paper (" Whatman " No. 1). Hexanoic and octanoic acid were also separately spotted on to the same paper, which was then exposed to ammonia gas to convert the acids into their ammonium salts. On chromatography of these salts with butanol–1·5N-ammonium hydroxide (1 : 1) as the mobile phase,[19] and the development of the paper with Bromothymol Blue, the mixed degradation acids separated into a large spot and a small spot, having R_F values identical with those of ammonium hexanoate (R_F 0·61) and octanoate (R_F 0·74), respectively.

Oxidation of the dilactone (XIII) under parallel conditions gave similar results.

The authors are indebted to Professor B. Lythgoe, F.R.S., for helpful discussions and information in advance of publication, Dr. J. B. Matthews of the Thornton Research Centre, Shell Refining Co. Ltd., for arranging the mass-spectral determination, and Mr. A. Quayle for performing these measurements and interpreting the results. They also thank the Department of Scientific and Industrial Research and Messrs. Goodlass Wall and Co. Ltd. for Maintenance Grants (to D. F. J. and A. D. G. P., respectively).

UNIVERSITY OF LIVERPOOL. [*Received, March 8th,* 1961.]

[19] Reid and Lederer, *Biochem. J.*, 1951, **50**, 60.
[20] Ohashi, Yamamura, Terahara, and Nakanishi, *Bull. Chem. Soc. Japan*, 1960, **33**, 1630.