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THE CONSTITUTIONS OF ATROVENETIN AND OF SOME RELATED HERQUEINONE DERIVATIVES

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Abstract—Deoxy*nor*herquеinone and atrovenetin have been shown to be identical. On the basis of spectral evidence these compounds, and related substances, have been characterised as substituted 9-hydroxyperinaphthenones. This conclusion has been confirmed by oxidative degradation to the appropriate naphthalic anhydrides as well as by further oxidation to nitrococussic acid. The constitution of an important oxidation product, $C_{15}H_{14}O_9N_2$, obtained by the action of nitric acid on atrovenetin has been established. On the basis of this and other evidence the constitutions of atrovenetin and certain of its transformation products have been elucidated.

ATROVENETIN, $C_{19}H_{18}O_6$, was isolated from *Penicillium atrovenetum* by Neill and Raistrick[1] who characterised it by a large number of derivatives and obtained several important degradation products. The morphologically closely related species, *P. Herquei* Bainier and Sartory, contains, as colouring pigments, the substances herqueinone, $C_{19}H_{17}O_6$ (OMe) and *nor*herqueinone, $C_{19}H_{18}O_7$,[2,3,4] and in view of the similarity in the empirical formulae some relationship was to be suspected. However, whereas atrovenetin shows aromatic stability herqueinone does not. Deoxyherqueinone, obtained by zinc and acetic acid reduction of herqueinone,[3] on the other hand, has this stability. By comparison of their respective triacetates (Experimental), it has now been shown[5] that deoxy*nor*herqueinone, obtained in a similar manner from *nor*herqueinone, is identical with atrovenetin. Data derived from the chemistry of herqueinone is thus pertinent to structural work on atrovenetin.

Prior knowledge[1] as to the functional groups of atrovenetin may be summarised as follows. Atrovenetin contains a minimum of four hydroxyl groups (formation of tetramethyl ethers). On the basis of a carbonyl absorption band in the infra-red at about 1630 cm^{-1} (Table 1) a further oxygen atom was probably present as an inert (hydrogen bonded) conjugated carbonyl group. Since the tetramethyl ethers were insoluble in alkali the remaining oxygen function was probably ethereal. Atrovenetin contains 2–3 C-Methyl groups (Kühn–Roth).

With the deoxy*nor*herqueinone-atrovenetin identity established further conclusions with regard to this ethereal oxygen may be reached. Since, on acid hydrolysis, herqueinone and *nor*herqueinone give xanthoherquein $C_{15}H_{12}O_7$ and *nor*xanthoherquein $C_{14}H_{10}O_7$, respectively, both of which are optically inactive, together with methyl *iso*propylketone[3,4], it must be concluded that *nor*xanthoherquein represents the atrovenetin nucleus whilst the expelled five-carbon fragment contains both the original ethereal oxygen atom and the asymmetric centre responsible for the optical

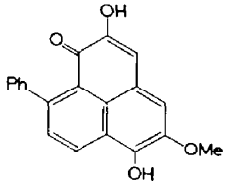
[1] K. G. Neill and H. Raistrick, *Biochem. J.* **65**, 166 (1957).
[2] F. H. Stodola, K. B. Raper and D. I. Fennell, *Nature, Lond.* **167**, 773 (1951).
[3] J. A. Galarraga, K. G. Neill and H. Raistrick, *Biochem. J.* **61**, 456 (1955).
[4] R. E. Harman, J. Cason, F. H. Stodola and A. L. Adkins, *J. Org. Chem.* **20**, 1260 (1955).

TABLE 1

Hydroxyperinaphthenones

Compound	Bands (cm^{-1}) in Nujol	
Atrovenetin (IX)	1620	1570
Perinaphthenone	1637 (1620)	1582
9-Hydroxyperinaphthenone	1625	1585
Xanthoherquein		1600 (broad)
Product obtained by $LiAlH_4$ redn. of (XII; R = H)	1630	1582
Product obtained by $LiAlH_4$ redn. of (XI; R = H)	1620	1577
Atrovenetin 'orange' trimethyl ether, (XII; R = H)	1613	1557
Atrovenetin 'yellow' trimethyl ether, (XI; R = H)	1607	1590
Atrovenetin tetramethyl ether A, (XII; R = Me)	1630	1577
Atrovenetin tetramethyl ether B, (XI; R = Me) or (XIII)	1613	1600

activity of atrovenetin. These conclusions have been substantiated. In particular, a close resemblance in ultra-violet spectra was apparent between the relevant derivatives of xanthoherquein, *nor*xanthoherquein, and atrovenetin (Table 2) suggesting the presence of a similar nucleus. The spectra also resembled the specially characteristic spectrum of 9-hydroxyperinaphthenone (Table 2).[5] At this time no naturally occurring perinaphthenone was known, but since then the isolation and characterisation of haemocorin[6] (I or equivalent tautomer) has been recorded.



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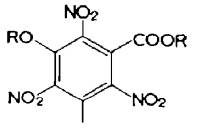

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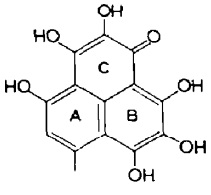

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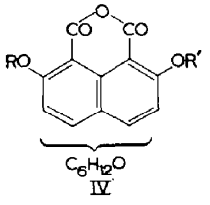

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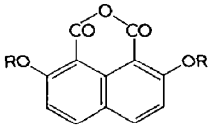


V

[5] D. H. R. Barton, P. de Mayo, G. A. Morrison, W. H. Schaeppi and H. Raistrick, *Chem. & Ind.* 552 (1956).
[6] R. G. Cooke and W. Segal, *Aust. J. Chem.* **8**, 107, 413 (1955); R. G. Cooke, Johnson and W. Segal, *Ibid.* **11**, 230 (1958).

TABLE 2

Compound	$\lambda\lambda_{max}$ (mμ) intensity (log ε) in parentheses						λmin. (mμ) intensity (log ϵ) in parentheses
9-Hydroxyperinaphthen-1-one	235 (4.38)	260 (3·92)	352 (4·26)	395(infl.) (3·68)	415 (3·95)	438 (4·05)	280 (2·97)
Deoxy*nor*herqueinone triacetate*	247(infl.) (4·28)	276 (4·19)	348–51 (4·07)	390(infl.) (4·00)	407–10 (4·18)	433 (4·27)	298 (2·81)
Atrovenetin triacetate†	247(infl.) (4·23)	275 (4·23)	347–50 (4·08)	390(infl.) (4·01)	409 (4·20)	433 (4·29)	298 (2·80)
Deoxyherqueinone diacetate	c.,250(infl.) (4·27)	278 (4·10)	358 (4·20)	389 (4·09)	410 (4·18)	433 (4·23)	294 (2·54)
Xanthoherquein tetracetate	240 (4·39)	267 (4·00)	375 (4·28)		420 (4·03)	445 (3·97)	292 (3·08)
*nor*Xanthoherquein pentacetate	242 (4·44)	266(infl.) (4·12)	370 (4·28)		415 (4·03)	440 (4·03)	298 (2·93)
Perinaphthenone		248 (4·34)	315 (3·58)	360 (4·02)	384 (3·95)		275 (2·95)
Atrovenetin, (IX)	222 (4·25)	250–260(infl.) (4·26)			385 (4·21)	410-20(infl.) (4·10)	310 (2·74)
Deoxyherqueinone, (XVII; R = H, R′ = Me) or (XVII; R = Me, R′ = H)		250–265(infl.) (4·26)			385(infl.) (4·22)	405	300 (3·17)
Xanthoherquein	216	255(infl.) (4·19)			395 (4·19)	410(infl.) (4·17)	305 (3·22)
Product obtained by $LiAlH_4$ reduction of (XI; R = H)′	220 (4·31)	239 (4·36)	272 (4·40)	340 (3·96)		422 (4·14)	300 (2·90)
Product obtained by $LiAlH_4$ reduction of (XII; R = H)′	220 (4·48)		275 (4·56)	342 (3·97)		438(4·27) 462(4·25)	295 (3·00)

* Additional inflection at 260–65 mμ log ε = 4·16
† Additional inflection at 265 mμ log ε = 4·15

With these facts in mind the degradation of xanthoherquein was attempted to confirm or disprove the hydroxyperinaphthenone nature of the nucleus. By heating on the steam bath with concentrated nitric acid nitrococussic acid (II; R = H)[7] was formed. This was characterised as the methyl ester methyl ether, and by decarboxylation[8] to 2:4:6-trinitro-*m*-cresol, the latter and its methyl ether being compared with authentic specimens. The genesis of (II; R= H) is most simply explained if two of the nitro groups are introduced by electrophilic decarboxylative nitration.[9] Its formation finds close analogy in the production of the same compound by the nitration of carminic acid.[7,8] If *nor*xanthoherquein is to be formulated as a perinaphthenone, then nitrococussic acid (II; R = H) must represent that ring which bears the methyl group and which has only one phenolic hydroxyl group. Since *nor*xanthoherquein has seven oxygen atoms attached to the nucleus it follows that every carbon atom in the nucleus must be attached to oxygen except the angular and central carbons and the C-Me, and the C-H (substituted to C-NO_2 in (II, R = H)) characterised in the formation of the nitrococussic acid. *nor*Xanthoherquein must, therefore, be (III) or equivalent tautomer. Nitrococussic acid was also obtained by similar oxidation of *nor*xanthoherquein and of atrovenetin.

[7] W. de la Rue, *Liebigs Ann.* **64**, 1 (1848).
[8] C. Liebermann and W. A. van Dorp, *Liebigs Ann.* **163**, 97 (1872).
[9] E. Grovenstein Jr., and U. V. Henderson, Jr., *J. Amer. Chem. Soc.* **78**, 569 (1956).

Our attention was next turned to the oxidative degradation of atrovenetin itself in an endeavour to determine whether the ether bridge was attached to ring C or ring B, (III). By very brief oxidation with alkaline hydrogen peroxide followed by chromatography on paper powder a substance, $C_{18}H_{16}O_6$, was obtained. This was optically active, could also be prepared by chromic acid oxidation of deoxyherqueinone and gave a diacetate on mild acetylation. This compound was assigned the part structure (IV; R = R′ = H) for the following reasons. First, the infra-red spectrum (Table 3) showed carbonyl bonds close to those found for 2:7-dihydroxynaphthalic anhydride (V; R = H), whilst those of the corresponding diacetate closely resembled the appropriate bonds in the spectrum of 2:7-diacetoxynaphthalic anhydride (V; R = Ac) and of naphthalic anhydride itself. Naphthalic anhydrides have, of course,[10] very characteristic infra-red bands. Secondly, the $C_{18}H_{16}O_6$ anhydride, its diacetate, and the authentic naphthalic anhydride derivatives all exhibited a strong blue fluorescence in ultra-violet light said to be characteristic of a C—O—C bridge in the peri-position of naphthalene.[11]

The remarkable difference between the position of the infra-red carbonyl bands in the anhydride and its diacetate, paralleled by a similar change between the spectrum of (V; R = H) and (V; R = Ac), is to be attributed to hydrogen bonding of hydroxyl and carbonyl groups on *both* sides of the anhydride ring as in (IV; R = R′ = H). That this was so was confirmed by a study of a product, $C_{19}H_{18}O_6$, obtained by oxidation of atrovenetin 'yellow' trimethyl ether[1] with chromium trioxide. This compound must be presented as (IV; R = Me; R′ = H) since it gave the naphthalic anhydride (IV; R = R′ = H) on demethylation with pyridine hydrochloride. By a similar oxidation of atrovenetin 'orange' trimethyl ether[1] a second methyl ether anhydride (IV; R = H; R′ = Me), which also gave (IV; R = R′ = H) on demethylation with pyridine hydrochloride, was obtained. The evidence for the orientation of the methoxyl groups in these anhydrides is presented later in the paper. The infra-red bands of the anhydrides from the 'yellow' and 'orange' trimethyl ethers are instructive (Table 3) and show clearly the hydrogen bonding effect of the *ortho*-hydroxyl group present in *both* isomers. It should be mentioned that the 'oxime' formed[1] by the anhydride from the 'yellow' trimethyl ether is now to be formulated as the derived N-hydroxyimide.[12]

The evidence so far presented restricts the attachment of the ethereal ring of atrovenetin to the two possibilities indicated in (VI) and (VII). A distinction between these two formulae was reached on the following evidence. By the nitric acid oxidation of atrovenetin Neill and Raistrick[1] obtained a phenolic product, $C_{15}H_{14}O_9N_2$, ($\lambda\lambda_{max}$ 263 and 344 mμ; log ε, 4·07 and 4·09 respectively) characterised by methylation with diazomethane to a monomethyl derivative. For this substance we propose the constitution (VIII; R = Me; R′ = R″ = H) on the following grounds.

First, the compound contains the moiety responsible for the nitrococussic acid formation, a simultaneous process in this degradation. Secondly, the structure adequately rationalises the stability of the molecule to such drastic oxidative conditions. Thirdly, the substance shows bands in the carbonyl region of the infra-red (Nujol) at 1785 (γ-lactone) and at 1740 (phthalide ring[13]) cm^{-1}. Fourthly, by treatment

[10] R. G. Cooke, *Chem. & Ind.* 142 (1955).
[11] H. E. French and J. E. Kircher, *J. Amer. Chem. Soc.* **66**, 298 (1944).
[12] D. E. Ames and T. F. Grey, *J. Chem. Soc.* 3518 (1955).
[13] F. D. Greene, *J. Amer. Chem. Soc.* **78**, 2250 (1956).

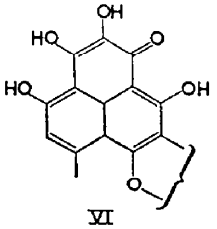
TABLE 3

Naphthalic anhydrides

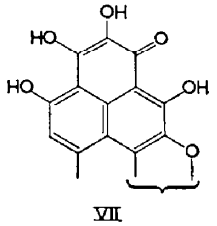
Compound	Bands (cm^{-1}) in Nujol	
Naphthalic Anhydride	1765	1735
2:7-Diacetoxynaphthalic anhydride (V; R = Ac)	1760 (broad)	1720
2:7-Dihydroxynaphthalic anhydride (V; R = H)	1720	1685
2:7-Dimethoxynaphthalic anhydride (V; R = Me)	1750	1720
*Anhydride (X; R = R′ = H)	1703	1660
Anhydride diacetate† (X; R = R′ = Ac)	1750	1720
Anhydride (X; R = Me, R′ = H) (from the 'yellow' trimethyl ether)	1750	1663
Anhydride (X; R = H, R′ = Me) (from the 'orange' trimethyl ether)	1747	1670

* Also in 1% $CHCl_3$ solution. (This indicates the absence of intermolecular hydrogen bonds.)
† This compound also showed absorption at 1773 cm^{-1} due to aromatic acetyloxy groups.

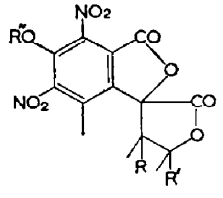
with base followed by methylation with diazomethane the phenol gave a methyl ether methyl ester. This showed bands in the infra-red (Nujol) at 1785 (γ-lactone) and at 1740 cm^{-1}. The latter band is to be attributed to the methyl ester and although high for a normal aromatic ester is to be compared with a band at 1740 cm^{-1} (in Nujol) found for methyl nitrococussate. Fifth, the structure (VIII) still retains the asymmetric carbon atom of atrovenetin and is compatible with the optical activity of the phenol. Finally, the ultra-violet spectrum of the phenol methyl ether (VIII; R = R″ = Me, R′ = H) was very similar to that of methyl nitrococussate methyl ether (II; R = Me, Experimental).



VI

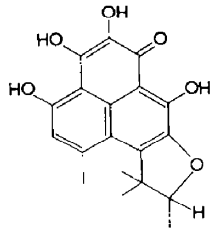


VII

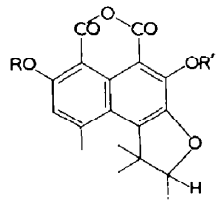


VIII

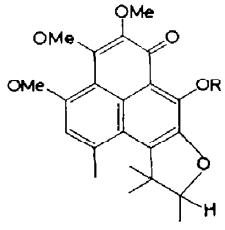
No evidence has yet been offered to distinguish between the alternative representations of the ether ring in (VIII). Thus in one formulation (VIII; R = Me; R′ = R″ = H) there is a hydrogen on the carbon atom to which the ethereal oxygen is attached, whilst in the other (VIII; R = R″ = H; R′ = Me) there is not. Dr. L. M. Jackman, to whom we express our best thanks, very kindly determined and interpreted the NMR spectrum of the phenol (VIII) in saturated pyridine solution (for calibration etc. see Experimental). There were signals at 1222 and 1218 (*gem.*-dimethyl) and 1134 (aromatic methyl) c.p.s. In addition there was a doublet (J = 6·5 c.p.s) at 1209 and a weak quartet (J = 6·5 c.p.s) at 1066 c.p.s. The 1066 c.p.s. signal is attributed to the proton (R′) attached to the same carbon atom as an oxygen atom and the doublet to the methyl group attached to this same carbon atom. The signal given by the proton is in accord with the structure (VIII; R = Me; R′ = R″ = H), its resonance frequency being close to the equivalent proton (1068) in *iso*propyl acetate.[14] Similarly the doublet at 1209 is close to the signal (1212 c.p.s.) for the methyl group of the ethyl residue of ethylacetate.[14] This evidence establishes the correctness of the formulation (VIII; R = Me; R′ = R″ = H).



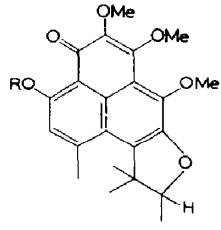
IX



X



XI



XII

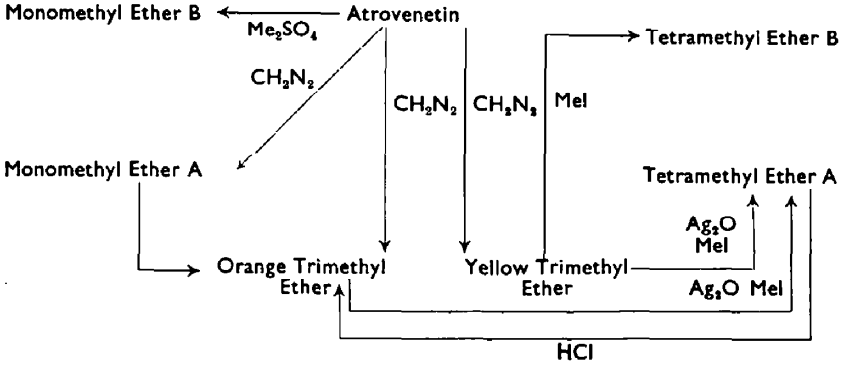
From these facts a unique structure for atrovenetin (IX or, of course, an equivalent tautomer) necessarily follows. The constitution (IX) accounts also for certain additional observations. By zinc dust distillation of atrovenetin Neill and Raistrick[1] obtained, in small yield, pyrene. On the basis of (IX) this is simply interpreted as cleavage of the ether followed by cyclisation onto the *peri*-position. The loss of methyl groups necessarily involved finds analogy in the chemistry of terramycin.[15]

With the structure of atrovenetin established it is now possible to discuss the

[14] A. A. Bothner-By, C. Naar-Colin and B. L. Shapiro, *NMR Spectra and Structure Correlations* Vol. II. Harvard Chemistry Department.

[15] F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *J. Amer. Chem. Soc.* **75**, 5455 (1953).

nature of certain of its transformation products. By the application of a number of methylation techniques Neill and Raistrick[1] prepared a large number of methyl ethers, the various interconversions being summarised below.

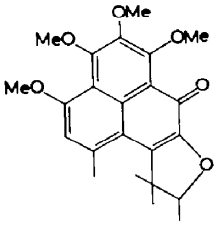


The nature of the trimethyl ethers has now been clarified by examination of their oxidation products. It has already been mentioned that chromic acid oxidation of the 'yellow' and 'orange' ethers gives two different anhydrides (IV; R or R′ = Me; R′ or R = H). In both of these one methoxyl group only is retained. It has now been found that nitric acid oxidation of the anhydride from the 'orange' trimethyl ether gives nitrococussic acid. This has been estimated by partition chromatography followed by spectroscopic analysis, and the substance responsible for the relevant band in the chromatogram has also been isolated as the crystalline acid and its identity confirmed. In contrast to this we were unable to isolate or detect any nitrococussic acid from the corresponding oxidation of the anhydride from the 'yellow' trimethyl ether. It thus follows that the anhydride from the 'orange' trimethyl ether is that in which the methoxyl group is *not* attached to the ring bearing the methyl group, and is therefore to be represented as (X; R = H; R′ = Me). The other anhydride then becomes (X; R = Me; R′ = H). Since, in the trimethyl ethers from which the anhydrides are derived, it must be presumed that the unmethylated hydroxyl group and the carbonyl group form a *peri*-hydrogen-bonded pair the yellow and orange trimethyl-ethers may be represented as (XI; R = H) and (XIII; R = H) respectively (or equivalent tautomers). Since tetramethyl ether B is derived from (XI; R = H), it seems probable that either (XI; R = Me) or (XIII) should represent this substance and similarly (XII; R = Me) or (XIV) must represent tetramethyl ether A. This is in accord with the hydrolysis of tetramethyl ether A back to the 'orange' trimethyl ether. Of these (XIV) must be correct because chromic acid oxidation of tetramethyl ether A gave the anhydride (X; R = H; R′ = Me), obtained from the 'orange' trimethyl ether. It was not, however, possible to distinguish between (XI; R = Me) and (XIII) in this way. The anomalous formation of *both* tetramethyl ethers from the yellow trimethyl ether requires the facile hydrolysis of one methoxyl group. This may be readily rationalised as an addition-elimination process, the hydrolysis taking place at a position β- or vinylogously β- to the carbonyl group.[16]

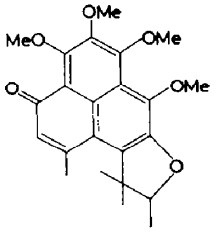
By the lithium aluminium hydride reduction of the trimethyl ethers of atrovenetin two isomeric substances, $C_{22}H_{24}O_5$, were obtained.[1] Since these both have the original

[16] Compare J. F. Grove, J. MacMillan, T. P. C. Mulholland and M. A. T. Rogers, *J. Chem. Soc.* 3949 (1952); A. E. Oxford, H. Raistrick and P. Simonart, *Biochem. J.* 33, 240 (1939).

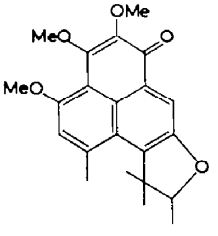
three methoxyl groups the reaction must be interpreted as a hydrogenolysis induced by 1:2- or 1:4-hydride addition followed by β-elimination. In confirmation of this both substances showed carbonyl bands (Table 1) indicative of a perinaphthenone. It follows that (XVa) or (XVb) and (XVIa) or (XVIb) may be written for the hydride



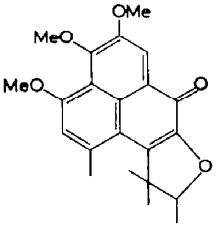
XIII



XIV



XVa



XVb

products derived from the 'yellow' and 'orange' trimethyl ethers respectively, but the available evidence does not permit a decision between the various structures.

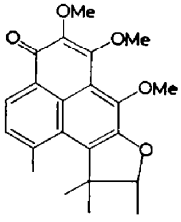
Since deoxyherqueinone gives the anhydride (IV; R = R′ = H), now to be represented as (X; R = R′ = H), on oxidation, with loss of the methoxyl group it follows that in this substance, and, therefore, in herqueinone itself, the methoxyl group must be situated in ring C. In addition methylation of deoxyherqueinone with a solution of diazomethane in methylene dichloride has been found to give atrovenetin 'orange' trimethyl ether (XII; R = H) whence it follows that deoxyherqueinone must be represented by (XVII; R = H; R′ = Me) or by (XVII; R = Me, R′ = H). Deoxyherqueinone is not identical with either of the atrovenetin monomethyl ethers.

The biogenesis of atrovenetin calls for some comment. The nucleus, as found in xanthoherquein, appears to be based on the same poly-β-diketone system as has been postulated for the genesis of the naturally occurring anthraquinones,[17] and related *m*-hydroxylated aromatic substances. The manner in which the coiling of the poly-β-diketone chain is envisaged is indicated in (XVIII). The ethereal side chain of atrovenetin is also very unusual and requires comment. Of aromatic natural products of established constitution,[18] containing an isoprene side chain the overwhelming majority have this unit attached to the aromatic nucleus by the terminal (C_1) carbon

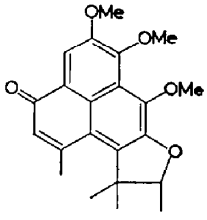
[17] *Inter al.* Sir R. Robinson, *Structural Relations of Natural Products.* Clarendon Press (1955); A. J. Birch and F. W. Donovan, *Aust. J. Chem.* **6**, 361 (1953).

[18] W. Karrer, *Konstitution und Vorkommen der Organischen Pflanzenstoffe.* Birkhauser Verlag, Basel (1958).

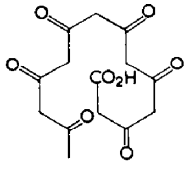
atom; that is through the carbon atom bearing the primary hydroxyl in a mevalonic acid precursor. These side chains also frequently contain an allylic double bond or its oxygenated equivalent. It is thus probable that these units are formed by elimination of the tertiary hydroxyl group of a mevalonic acid precursor followed by a



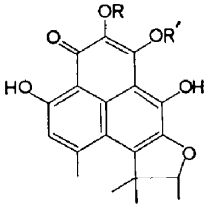
XVIa



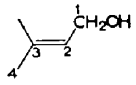
XVIb



XVIII



XVII



XIX

XX

substitution process on the primary hydroxyl carbon. An intermediate such as (XIX or equivalent) may therefore be involved. The ether bridge in atrovenetin and that in dunnione[19] may thus be formed from the alternative coupling site in the mesomeric cation (XX) obtainable from (XIX).

We report briefly in the Experimental section the preparation of monobromo-derivatives of xanthoherquein, atrovenetin, herqueinone and *iso*herqueinone. The formation of such mono-derivatives from xanthoherquein and atrovenetin is, of course, to be expected since one free aromatic position *ortho* to a phenolic hydroxyl is available in both compounds. The studies now reported on atrovenetin have considerable bearing on the structure of herqueinone. We defer, however, any speculation pending the completion of experiments which are now in hand.

EXPERIMENTAL

$[\alpha]_D$ are in $CHCl_3$, ultra-violet absorption spectra refer to EtOH solution. Infra-red spectra were kindly determined by Dr. G. Eglinton and his colleagues (Glasgow). Microanalyses are by Mr. J. M. L. Cameron (Glasgow) and Miss J. Cuckney (Imperial College) and their colleagues. M.ps. were determined on the Köfler block unless otherwise stated. The term light petroleum refers to the fraction of b.p. 60–80°. The NMR spectra were determined on a Varian nuclear resonance spectrometer using a 40 Mc oscillator. The spectra were calibrated against *cyclo*hexane as an internal standard using the usual side band technique. *cyclo*Hexane at infinite dilution in CCl_4 gives a single line at 1204 c.p.s. relative to external toluene, the aromatic line of the latter being given the arbitary value of 1000 c.p.s.

[19] J. R. Price and Sir R. Robinson, *J. Chem. Soc.* 1523 (1939); 1493 (1940); R. G. Cooke, *Aust. J. Sci. Res.* 3A, 481 (1950).

Bromoherqueinone

Herqueinone (50 mg) in acetic acid (100 ml) was treated with a solution of bromine in the same solvent (1·03 N; 1 ml). Titration of an aliquot indicated the uptake of 0·97 mole bromine in 10 min, no further uptake occurring over a further hour. Isolation of the product in the usual way gave, after crystallisation from chloroform—light petroleum *bromoherqueinone*, m.p. 235° (decomp), $[\alpha]_D$ +460° (*c* 0·054), $\lambda\lambda_{max}$ 222, 254–57, 266, 322–323, 410–414 mμ (log ε 4·49, 4·25, 4·27, 4·53, 3·72 respectively) (Found: C, 52·95; H, 4·55; Br, 17·5; OMe, 6·5; C—Me, 7·35; $C_{20}H_{19}O_7Br$ requires: C, 53·25; H, 4·25; Br, 17·7; OMe, 6·9; 2C—Me 6·65%).

Bromoisoherqueinone

A solution of bromine in acetic acid (0·88 N; 1 ml) was added to *iso*herqueinone (102 mg) dissolved in acetic acid (40 ml). The titration of aliquots indicated the uptake of 0·95 mole bromine. The red needles separating from the reaction medium were collected and crystallised from chloroform-light petroleum to give *bromoisoherqueinone*, decomposing slowly from 240° to 260° (capillary), $[\alpha]_D$ O° (*c* 0·98). (Found: C, 53·35; H, 4·55; Br, 18·1. $C_{20}H_{19}O_7Br$ requires: C, 53·25; H, 4·25; Br, 17·7%). The infrared spectrum showed small differences from that of bromoherqueinone in the fingerprint region.

Bromoxanthoherquein

Xanthoherquein (53 mg) in acetic acid (100 ml) was treated with a solution of bromine in acetic acid (1.0 N; 1 ml). The titration of aliquots indicated the cessation of uptake after the consumption of 1·3 moles bromine. Isolation of the product in the usual way, followed by crystallisation from acetic acid, gave bright yellow needles of *bromoxanthoherquein*, decomposing slowly above 200°, $\lambda\lambda_{max}$ 220 and 426–429 mμ (log ε 4·64 and 4·23 respectively) (Found: C, 46·8; H, 3·05; Br, 20·65. $C_{15}H_{11}O_7Br$ requires: C, 47·05; H, 2·9; Br, 20·85%).

Bromoatrovenetin

Atrovenetin (51 mg) in acetic acid (50 ml) was treated with a solution of bromine in acetic acid (0·1 N, 5 ml). The titration of aliquots indicated that uptake of bromine ceased after 40 min with the consumption of 1 mole. On being set aside overnight the product crystallised from the solution to give, after crystallisation from acetic acid, *bromoatrovenetin*, m.p. 232–5°. (Found: C, 54·45; H, 4·35; Br, 19·4. $C_{19}H_{17}O_6Br$ requires: C, 54·15; H, 4·05; Br, 18·9%).

Deoxyherqueinone diacetate

(a) Herqueinone (520 mg) in acetic acid (125 ml) was shaken with zinc dust (5 gm) in a nitrogen atmosphere for 20 min. After filtration the acetic acid was removed *in vacuo* and the residue acetylated in the usual way (acetic anhydride-pyridine) to give, after crystallisation from methanol, *deoxyherqueinone diacetate*, m.p. 174–175°, $[\alpha]_D$ −57° (*c* 0·46). (Found: C, 65·25; H, 5·65; Ac, 20·2; $C_{24}H_{24}O_8$ requires: C, 65·45; H, 5·5; 2Ac, 19·5%).

(b) *iso*Herqueinone (110 mg) in acetic acid (25 ml) was shaken with zinc dust (1.0 g) under nitrogen for 20 min. Treatment of the product as described under section (a) gave deoxyherqueinone diacetate (m.p. and mixed m.p.).

Deoxynorherqueinone triacetate (Atrovenetin triacetate)

*nor*Herqueinone (354 mg) in pyridine (12 ml) and acetic acid (30 ml) was shaken with zinc dust (3 g) for 30 min. The reaction mixture was poured into water and the product isolated with chloroform. The amorphous residue remaining after removal of the solvent was a complex containing nitrogen. This was washed with acetone and used without further purification for acetylation. The deoxy*nor*herqueinone complex (298 mg) was set aside overnight with pyridine (2 ml) and acetic anhydride (4 ml), slow dissolution occurring. Isolation of the product in the usual way gave deoxy*nor*-herqueinone triacetate; m.p. 185–187° (from methanol), $[\alpha]_D$ +70° (*c* 0·57). (Found: C, 63·75; H, 5·4; Ac, 27·4. Calc. for $C_{25}H_{24}O_9$: C, 64·1; H, 5·15; 3Ac, 27·55%). The m.p. of the compound was undepressed on admixture with atrovenetin triacetate m.p. 185–7°, $[\alpha]_D$ +73° (*c* 0·70). The ultra-violet spectra (Table 2) were superposable as were the infra-red spectra both in nujol and chloroform. Powder diffraction photographs were kindly taken by Dr. J. C. Speakman (Glasgow) using CuK α-radiation and a 9·0 cm diameter camera and confirmed this identity.

Nitric acid oxidation of xanthoherquein

(a) Xanthoherquein (120 mg) and conc nitric acid (5 ml) were heated together on the steam bath for one hour. The mixture was then poured into water and the product isolated with ether. After removal of the solvent the residue was filtered in ether-benzene solution (1:9) through a short column (3 g) of silica. Crystallisation from benzene-light petroleum gave nitrococussic acid (II; R = H)[7,8] m.p. 179–180° (evacuated capillary), λ_{max} 380–383 mμ (log ε 3·9) (Found: C, 33·2; H, 2·0; N, 14·3. Calc. for $C_8H_5N_3O_9$: C, 33·45; H, 1·75; N, 14·65%). The compound gave a yellow-brown colour with ferric chloride and showed bands at 3480 and 1720 cm^{-1} in the infra-red spectrum.

(b) Xanthoherquein (112 mg) was heated on the steam bath with conc nitric acid (5 ml) for 10 min. Isolation of the product as described under section (a) followed by methylation with excess ethereal diazomethane gave, after filtration in light petroleum solution through a short column (2 g) of alumina and crystallisation from aqueous methanol, *dimethyl nitrococussate*, (II; R = Me) m.p. 135–6°, λ_{max} 298–301 mμ (log ε 3·24) (cf VIII; R = R″ = Me, R′ = H, λ_{max} 309 mμ (log ε, 3·35)) (Found: C, 38·1; H, 3·3; N, 13·0; OMe, 19·25. $C_{10}H_9O_9N_3$ requires: C, 38·1; H, 2·9; N, 13·3; 2 (OMe) 19·8%). The material gave no colour with ferric chloride and showed a band in the infra-red spectrum at 1740 cm^{-1}. Decarboxylation of nitrococussic acid, as described by Liebermann and van Dorp,[8] gave 2:4:6-trinitro-*m*-cresol, m.p. 104–106°, undepressed on admixture with an authentic specimen of the same m.p.[20] The ultra-violet and infra-red spectra of the decarboxylation product and of the authentic 2:4:6-trinitro-*m*-cresol were superposable. Methylation of the decarboxylated product with excess ethereal diazomethane gave, after crystallisation from methanol, the methyl ether, m.p. 89–90°. (Found: N, 16·05. Calc. for $C_8H_7O_7N_3$: N, 16·35%). Preparation of the methyl ether of 2:4:6-trinitro-*m*-cresol gave material identical in every respect with the above-mentioned methyl ether.

Nitric acid oxidation of nor*xanthoherquein*

*nor*Xanthoherquein (235 mg) was heated on the steam bath for 70 min with conc nitric acid (10 ml). Isolation of the product in the manner described in the oxidation

[20] E. Reisz and F. Pilpel, *Monatshefte* **50**, 335 (1928).

of xanthoherquein gave material, m.p. 180–181 (decomp, sealed capillary) undepressed on admixture with nitrococussic acid. Methylation with ethereal diazomethane gave material m.p. 134–5°, undepressed on admixture with the methylation product of nitrococussic acid.

The anhydride (X; R = R′ = H)

(a) Atrovenetin (100 mg) was dissolved in boiling absolute alcohol (100 ml), the solution cooled to O° and sodium hydroxide solution (4 N; 18 ml) added, followed rapidly by hydrogen peroxide (30%; 18 ml) and water (20 ml). After standing 5 min at 0° the excess peroxide was destroyed with platinum catalyst, and hydrochloric acid (6 N) added to adjust the pH to 3–4. The mixture was then diluted with saturated ammonium chloride solution and repeatedly extracted with ether. Evaporation of the ether gave a red oily residue. This was applied to a cellulose[21] column (35 cm × 4 cm) in, and eluted with, the upper layer of the system obtained from *n*-butanol, benzene and aqueous ammonium carbonate and hydroxide, 1·5 N with respect to each (1 : 49 : 50). The zone which fluoresced blue under ultra-violet light was collected, and after removal of the solvent the residue was crystallized from benzene-light petroleum and chloroform-light petroleum to give the *anhydride* (X; R = R′ = H), m.p. 255–256°, $[\alpha]_D$ + 76°(*c* 0·17), $\lambda\lambda_{max}$ 256, 297–298, 360–361 mμ (log ε 4·47, 3·94, 4·12 respectively) (Found: C, 65·9; H, 5·0; $C_{18}H_{16}O_6$ requires: C, 65·85; H, 4·9%). The compound did not react with sodium hydrogen carbonate but gave a deep brown-red colour with ethanolic ferric chloride. A negative result was obtained in the Thomas catechol test.[22] Solutions of the compound gave a bright blue fluorescence under ultra-violet light. Acetylation of the compound (acetic anhydride-pyridine) gave, after isolation and crystallisation from ethyl acetate-light petroleum, the *diacetate* (X; R = R′ = Ac), m.p. 176–181°, $\lambda\lambda_{max}$ 264 and 377–378 mμ (log ε 4·59 and 4·07 respectively) (Found: C, 64·35; H, 4·85; Ac, 20·6; $C_{22}H_{20}O_8$ requires: C, 64·05; H, 4·9; 2Ac, 20·85%).

(b) Herqueinone (292 mg) was dissolved in pyridine (5 ml) and acetic acid (25 ml) and after the addition of zinc dust (3 g) the mixture was shaken for 20 min. The zinc was then removed by filtration, the mixture acidified with 6 N sulphuric acid and the precipitated zinc salts filtered off. The filtrate was heated on the steam bath, and a solution of chromic acid (590 mg) in aqueous acetic acid (85%; 23 ml) added dropwise over 30 min. Heating was continued over 1½ hr. After working up in the usual way the product was chromatographed on silica, followed by crystallisation from chloroform–light petroleum, to give the anhydride (X; R = R′ = H), m.p. and m.m.p. 250–252°, $[\alpha]_D$ +72° (*c* 0·18). The ultra-violet and infra-red spectra were superposable.

(c) The anhydride (X; R = Me; R′ = H) (36 mg) was added to refluxing pyridine hydrochloride (1.0 g) and the heating continued for 4 min. The residue obtained after isolation in the usual way, followed by evaporation of the solvent, was dissolved in benzene and filtered through a short column of silica to give the anhydride (X; R = R′ = H), m.p. and m.m.p. 254–255°, $[\alpha]_D$ +73° (*c* 0·51). The identity was confirmed by the identical infra-red spectra of the two samples.

(d) The anhydride (X; R = H; R′ = Me) (31 mg) was demethylated as described for the anhydride (X; R = Me; R′ = H) under (c) to give, after crystallisation from chloroform–light petroleum, the anhydride (X; R = R′ = H), m.p. and mixed m.p.

[21] L. Hough, J. K. N. Jones, and W. H. Wadman, *J. Chem. Soc.* 2511 (1949).
[22] A. A. Thomas, *Chim. Anal.* **29**, 15 (1947).

255–256°, $[\alpha]_D$ +78° (*c* 0·56). The identity was confirmed by the superposability of the ultra-violet and infra-red spectra.

The anhydride (X; R = H; R′ = Me)

(a) Atrovenetin 'orange' trimethyl ether (510 mg) was dissolved in acetic acid (3 ml) at 100°. Chromium trioxide (572 mg) in aqueous acetic acid (85%; 23 ml) was added dropwise and the heating continued for 2 hr after the addition. After isolation in the usual way the product was added in benzene solution to a column of silica (30 g). Elution with ether-benzene (1 : 99) gave, after crystallisation from chloroform-light petroleum, the *anhydride* (X; R = H; R′ = Me), m.p. 235–236°, $[\alpha]_D$ +75° (*c* 0·58). [The mixed m.p. with the anhydride (X; R = Me; R′ = H) was 212°], $\lambda\lambda_{max}$ 233, 258, 341, 370 and 386 mμ (log ε, 4·26, 4·51, 4·07, 4·11 and 4·07 respectively) (Found: C, 66·5; H, 5·5; OMe, 9·15; $C_{19}H_{18}O_6$ requires: C, 66·65; H, 5·3; 1 OMe, 9·05%).

(b) Atrovenetin tetramethyl ether A (99 mg) was dissolved in acetic acid (2 ml) at 100°, and chromium trioxide (110 mg) in aqueous acetic acid (85%; 6 ml) added dropwise. Heating was continued for 2 hr. Isolation of the product as described under (a) gave, after crystallisation from chloroform-light petroleum, the anhydride (X; R = H; R′ = Me), identical in every respect with that obtained by oxidation of the 'orange' trimethyl ether.

Nitric acid oxidation of atrovenetin

Atrovenetin (103 mg) was heated on the steam bath for 2 hr with conc nitric acid (5 ml). Isolation of the product in the usual way gave an oil which was methylated with excess ethereal diazomethane and the methylated derivative, in benzene-light petroleum solution (7 : 3), filtered through a short column of alumina. Re-chromatography on alumina gave methyl nitrococussate, m.p. and m.m.p. 133–135°.

Nitric acid oxidation of the anhydrides (X; R = Me; R′ = H) *and* (X; R = H; R′ = Me)

(a) The anhydride (X; R = Me; R′ = H) and the anhydride (X; R = H; R′ = Me) (20·8 mg) were each heated for 1 hr with conc nitric acid (1 ml) on the steam bath. After isolation of the products in the usual way, the yellow gums so obtained were examined by ascending partition chromatography using the upper layer of the system *n*-butanol-acetic acid-water (4 : 1 : 5). The section of the strips in the region R_F 0·4 (that of nitrococussic acid) was cut into strips and eluted with boiling ethanol. With an appropriate blank, and using the optical density at 380 mμ it was found that the anhydride (X; R = H; R′ = Me) gave >200 times as much nitrococussic acid as did the anhydride (X; R = Me; R′ = H).

(b) The anhydride (X; R = H; R′ = Me) (123 mg) was heated on the steam bath with conc nitric acid (6 ml) for 1 hr. The gum obtained by isolation in the usual way was added in *iso*-propanol-ammonia (0·15 N) (4 : 1) to a column of cellulose powder (21 cm × 2·5 cm) previously washed with ethanol and with *iso*propanol-ammonia. The crystalline fractions were combined and recrystallised from chloroform-light petroleum to give nitrococussic acid, identified by m.p., mixed m.p., ultra-violet and infra-red spectra.

(c) The anhydride (X; R = Me; R′ = H) (160 mg) was oxidised on the steam bath in conc nitric acid (5 ml) for 1 hr. Isolation of the product gave a gum which

crystallised on titration with methanol. Crystallisation from chloroform-light petroleum gave a *compound*, m.p. 215–225° (gas evolution), $[\alpha]_D$ +161° (*c* 0·29), $\lambda\lambda_{max}$ 231 and 321 mμ (log ε, 4·50 and 3·74 respectively). (Found: C, 52·9; H, 4·45; N, 3·3; OMe, 7·2; $C_{18}H_{17}O_{10}N$ requires: C, 53·05; H, 4·2; N, 3·45; 1 OMe, 7·6%).

Base treatment of the phthalide (VIII; R = Me; R′ = R″ = H)

The phthalide (VIII; R = Me; R′ = R″ = H) (104 mg) in ethanol (5 ml) was added to water (5 ml) containing sodium hydroxide solution (4 N; 30 drops). After 10 min the solution was acidified (hydrochloric acid) and the product isolated with ether. The yellow gum obtained on evaporation of the ether was methylated, in ethyl acetate solution, with excess ethereal diazomethane. Evaporation and crystallisation from dry ether gave the *methyl ester methyl ether*, m.p. 125–130°, $[\alpha]_D$ −89° (*c* 1·08), λ_{max} 295 mμ (log ε 3·23) (Found: C, 49·75, H, 5·0; N, 6·65; OMe, 15·05; $C_{17}H_{20}O_{10}N_2$ requires: C, 49·5; H, 4·9; N, 6·8; 2 OMe, 15·05%).

2:7-*Dimethoxynaphthalic anhydride* (V; R = Me)

3:8-Dimethoxyacenaphthene quinone[23] (504 mg) was dissolved in ethanol (3 ml) and sodium hydroxide solution (4 N; 25 ml), hydrogen peroxide (30%; 25 ml) and water (35 ml) added. After standing 30 min the solution was acidified and the crystalline precipitate collected. Crystallisation from ethylene glycol then gave 2:7-*dimethoxynaphthalic anhydride* (V; R = Me), m.p. 340–344°, $\lambda\lambda_{max}$ 245, 283 and 365 mμ (log ε, 4·58, 3·65 and 4·26 respectively) (Found: C, 65·05; H, 4·05; OMe, 23·9. $C_{14}H_{10}O_5$ requires: C, 65·1; H, 3·9; 2 OMe, 24·05%).

2:7-*Dihydroxynaphthalic anhydride* (V; R = H)

2:7-Dimethoxynaphthalic anhydride (40 mg) was added to refluxing pyridine hydrochloride (1·2 g) and the heating continued for a further 3 min. The product was isolated in the usual way and crystallised from chloroform-light petroleum to give 2:7-*dihydroxynaphthalic anhydride* (V; R = H), m.p. 283–284°, $\lambda\lambda_{max}$ 225, 240, 285, 335, 355, and 365 mμ (log ε 4·39, 4·39, 3·73, 3·97, 4·03 and 4·09 respectively) (Found: C, 62·4; H, 2·85. $C_{12}H_6O_5$ requires: C, 62·6; H, 2·65%). The anhydride (37 mg) on acetylation (acetic anhydride-pyridine) gave, in the usual way, after crystallisation from chloroform-light petroleum, the *diacetate* (V; R = Ac) m.p. 265–269° (sealed capillary) $\lambda\lambda_{max}$ 238, 330 and 343 (log ε, 4·64, 4·09 and 4·11 respectively) (Found: C, 60·85; H, 3·4. $C_{16}H_{10}O_7$ requires: C, 61·15; H, 3·2).

Dimethyl 2:7-*dimethoxynaphthalate*

2:7-Dimethoxynaphthalic anhydride (55 mg) was dissolved in aqueous sodium hydroxide (4 N; 1·5 ml) and water (9·5 ml). The solution was acidified at 0° and the precipitate collected immediately. The wet precipitate was suspended in methylene dichloride and methylated with ethereal diazomethane. Isolation of the product and crystallisation from chloroform-light petroleum gave *dimethyl* 2:7-*dimethoxynaphthalate*, m.p. 128–130°, $\lambda\lambda_{max}$ 239, 308–13, 320, and 334 mμ (log ε, 4·79, 3·74, 3·74, 3·74 respectively) (Found: C, 62·75; H, 5·05; OMe, 40·7; $C_{16}H_{16}O_8$ requires: C, 63·15; H, 5·3; (4) OMe, 40·8%).

[23] H. Staudinger, H. Goldstein, and E. Schlenker, *Helv. Chim. Acta* **4**, 342 (1921).

Methylation of deoxyherqueinone

Deoxyherqueinone (153 mg) was treated in methylene dichloride with an excess of diazomethane in methylene dichloride solution and set aside for 20 hr. The red oil obtained on evaporation of the solvent was chromatographed on silica to give, after crystallisation from chloroform-light petroleum, atrovenetin orange trimethyl ether, m.p. 176–179°, identical in all respects with an authentic specimen. The m.p. was depressed to 135° on admixture with the yellow trimethyl ether (m.p. 163-169°).

Acknowledgment—We thank the Government Grants Committee of the Royal Society and Imperial Chemical Industries Limited for financial assistance. One of us (G. A. M.) acknowledges the award of a D.S.I.R. Maintenance Grant. We are glad to thank Dr. W. H. Schaeppi for the preparation of and measurement of the ultra-violet absorption spectra of various derivatives of herqueinone.[5]