Tetrahedron Vol 24. pp 199 to 229 Pergamon Press, 1968 Printed in Great Britain

THE STRUCTURES OF ANISATIN AND NEOANISATIN
TOXIC SESQUITERPENES FROM *ILLICIUM ANISATUM* L.[1]

K. YAMADA, S. TAKADA, S. NAKAMURA and Y. HIRATA
Chemical Institute, Faculty of Science, Nagoya University, Chikusa, Nagoya, Japan

(*Received in Japan* 24 *February* 1967; *accepted for publication* 14 *April* 1967)

Abstract—Two toxic compounds, anisatin (I) and neoanisatin (XXV) were isolated from *Illicium Anisatum* L. Extensive studies on noranisatin (II), an oxidation product of I were carried out by chemical and spectral methods. Two dihydrocoumarins (XI, XII) were obtained by stepwise degradation of II, the structures of which were firmly established. On the basis of the structures of XI and XII together with spectral evidence of important derivatives such as VII, VIII, IX and XV, the structure of II was determined as IIk. Based on the structure of II, anisatin was represented as If, which contains a stable β-lactone. The structure of neoanisatin was established as XXVa by a variety of oxidation reactions: a common oxidation product VII was obtained from both natural products, I and XXV. Some of the reactions of special interest are illustrated.

INTRODUCTION

THE convulsant activity of the seeds of *Illicium Anisatum* L. (Japanese star anise; Japanese name, *Shikimi*) has been known[2] for several centuries. In spite of the efforts of many workers,[3] attempts to isolate the active principle were fruitless, until in 1952 Lane *et al.*[4] reported the isolation of a pure toxic compound, anisatin with a molecular formula, $C_{15}H_{20}O_8$, m.p. 215–220° and the partial structure shown below.

$$(CH_3)_2\cdot(C_{11}H_9)\cdot(OH)_5\cdot(\text{—CO—O—CO—})$$

The present paper describes the whole structure including the stereochemistry of anisatin. During the isolation of anisatin we obtained another toxic compound, neoanisatin $C_{15}H_{20}O_7$, m.p. 237–238°. The relative amounts of anisatin and neoanisatin varied with the date of collection of the seeds. Neoanisatin is as toxic as anisatin; the intraperitoneal dose for mice is ca. 1γ/g of the body weight. The structure determination of neoanisatin has also been accomplished and is reported in the latter part of this paper.

ANISATIN

The molecular formula, $C_{15}H_{20}O_8$ of anisatin (I) presented by Lane's group was confirmed by elemental analysis and mass spectrometry (M^+, 328). Difficulties were encountered in the degradation studies of anisatin itself because of the frequent formation of ill-defined, amorphous derivatives in addition to the poor solubility of anisatin in common organic solvents such as benzene, ether and chloroform. In contrast, noranisatin (II), an oxidation product of anisatin afforded derivatives, which could be well characterized. Thus, the efforts of the structural elucidation were initially directed to noranisatin (II) and then to anisatin (I).

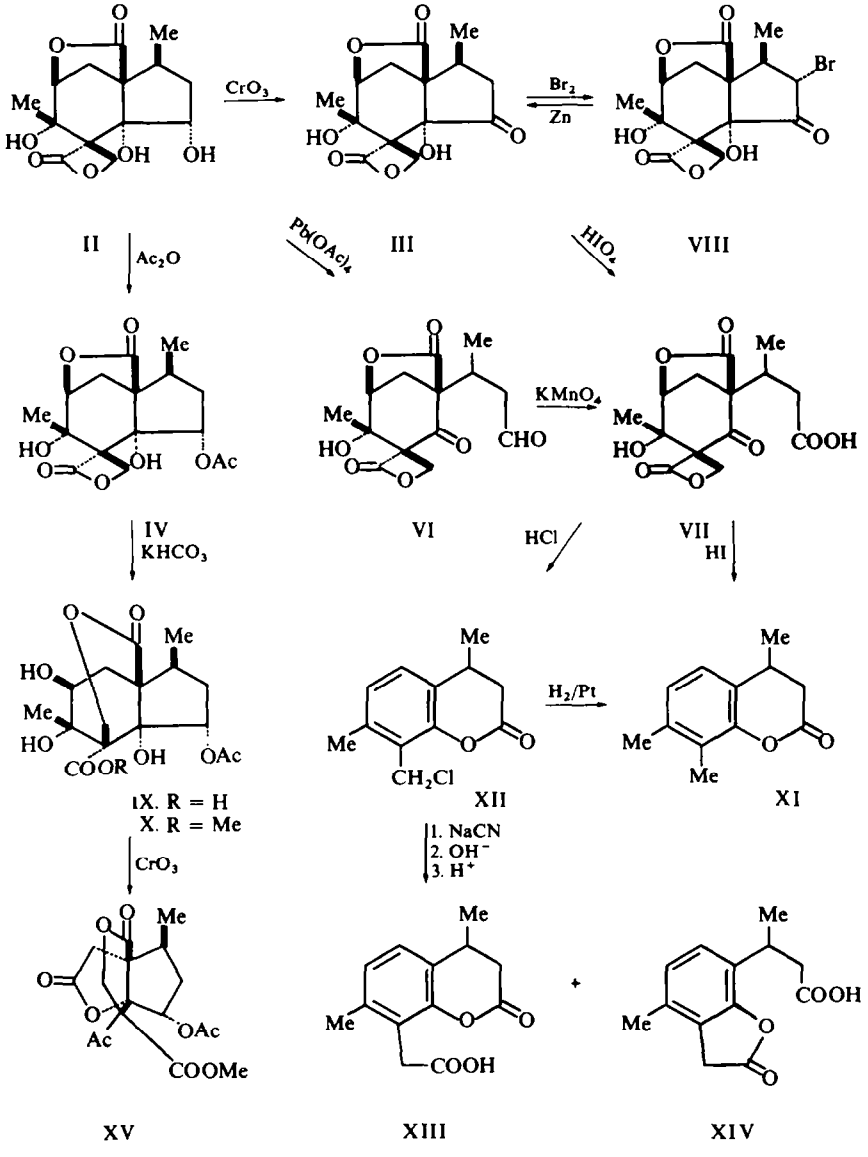
(1) *Structure of noranisatin, an oxidation product of anisatin*

(a) *The presence of a* 1,2*-glycol on a* 5*-membered ring.* On oxidation with potassium

permanganate in acetic acid, anisatin gave two neutral compounds, noranisatin (II), $C_{14}H_{18}O_7$ and noranisatinone (III), $C_{14}H_{16}O_7$. Noranisatinone (III) was also obtained by chromic acid oxidation of II in acetic acid. Main derivatives of noranisatin (II) are depicted in Scheme I.

Noranisatin (II) showed two carbonyl bands (1832, 1778 cm^{-1} in chloroform) and exhibited no UV absorption. The NMR spectrum (Fig. 1) of II indicated the presence of a secondary Me group (3H, 1·03 ppm, doublet, $J = 6·5$ c/s), a tertiary Me group (3H, 1·50 ppm, singlet) and three OH groups whose signals disappeared by hydrogen–deuterium exchange on addition of deuterium oxide.

Scheme I. Derivatives of noranisatin (II)



Noranisatin consumed one mole of periodic acid in aqueous methanol slowly and lead tetraacetate in acetic acid rapidly. Noranisatin formed a monoacetate (IV) $C_{16}H_{20}O_8$ with acetic anhydride–pyridine and a carbonate (V) $C_{15}H_{16}O_8$ with phosgene in tetrahydrofuran–pyridine, whose IR spectrum (KBr) showed a band at 1798·cm^{-1} due to a 5-membered ring carbonate, in addition to two carbonyl bands (1830, 1770 cm^{-1}) originally present in noranisatin (II). From the above findings, a 1,2-glycol group is present in II.

Lead tetraacetate oxidation of noranisatin (II) afforded noranisatin ketoaldehyde (VI) $C_{14}H_{16}O_7$, whose IR spectrum (KBr) showed two new carbonyl bands (an

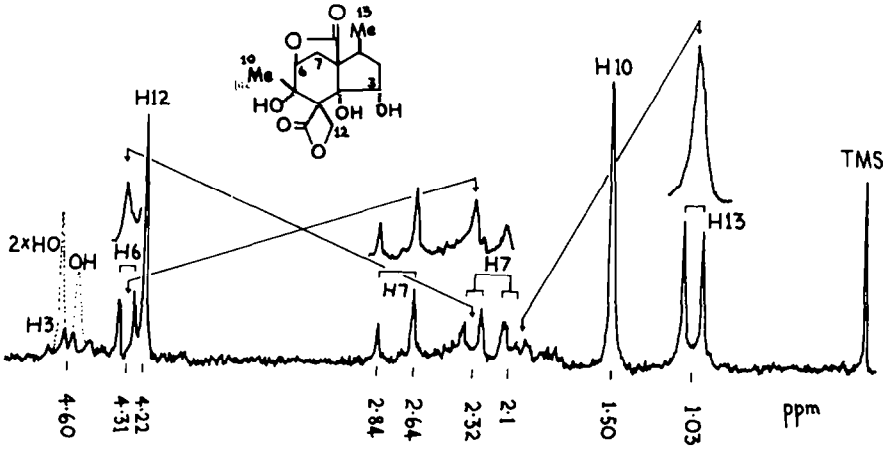
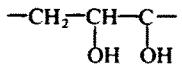


FIG. 1. The NMR spectrum with spin-decoupled patterns of noranisatin (II): ppm from internal TMS at 60 Mc in deuteriochloroform.

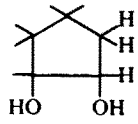
aldehyde and a ketone) at 1717 and 1700 cm^{-1}, in addition to two original carbonyl bands (1845, 1768 cm^{-1}). The ketoaldehyde VI, when treated with silver oxide was recovered unchanged but yielded noranisatin ketoacid (VII) $C_{14}H_{16}O_8$ on oxidation with potassium permanganate in dilute sulfuric acid–acetic acid. Formation of the ketoacid (VII) from noranisatin (II) via the ketoaldehyde (VI) indicates that OH groups of the 1,2-glycol are secondary and tertiary. The monoacetate IV was resistant to lead tetraacetate oxidation, indicating that the secondary OH group of the 1,2-glycol was acetylated. A signal at 4.60 ppm (1H, quartet, J = 5·5, 8·5 c/s) in the NMR spectrum of noranisatin (II) corresponds to a signal at 5·67 ppm (1H, quartet, J = 7·0, 9·5 c/s) in the NMR spectrum of its monoacetate (IV). This signal was assigned to a hydrogen on carbon bearing the acetoxyl group in the monoacetate (IV) and the multiplicity (quartet) of the signal showed the presence of two hydrogens on carbon adjacent to the carbon atom carrying the acetoxyl group. From the above results, the partial structure of noranisatin is represented by IIa. The ketoacid (VII) prepared from noranisatin (II) via the ketoaldehyde (VI) was also obtained by periodic acid oxidation of noranisatinone (III), indicating that the secondary OH group of the 1,2-glycol in noranisatin (II) was oxidized to a ketone in noranisatinone (III), whose carbonyl band appeared at 1754 cm^{-1}. This finding suggests that the 1,2-glycol is attached to a 5-membered ring; the partial structure then of noranisatin

is represented by IIb. The partial structure IIb is further confirmed by the formation of dihydrocoumarins from the ketoacid (VII), which will be described later.

(b) *Nature of all hydrogens of noranisatin.* Bromination of noranisatinone (III) in acetic acid afforded bromonoranisatinone (VIII) $C_{14}H_{15}O_7Br$, carbonyl bands of which appeared at 1833, 1778, 1770 cm^{-1} (chloroform) in the IR spectrum. The



IIa



IIb

shift of the carbonyl band of the 5-membered ring ketone to a higher frequency (1754 cm^{-1} in III, 1770 cm^{-1} in VIII) suggested that an α-carbon of the ketone group was brominated, which was confirmed by regeneration of noranisatinone (III) on treatment of the bromo derivative VIII with zinc and acetic acid. The nature of all the hydrogens of the bromo compound VIII was clearly disclosed from the NMR spectrum (Fig. 2). The presence of a secondary Me group (3H, 1·25 ppm, doublet,

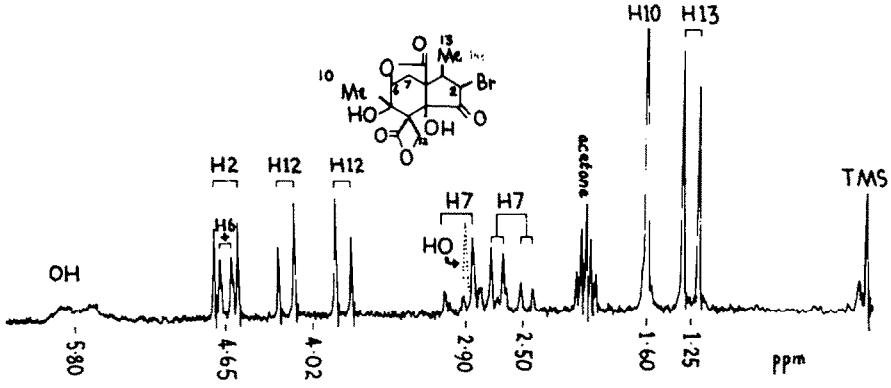
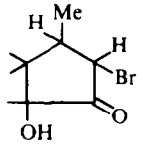


FIG. 2. The NMR spectrum of bromonoranisatinone (VIII): ppm from internal TMS at 60 Mc in deuterioacetone.

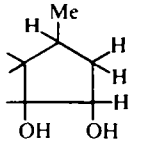
J = 7·0 c/s) and a tertiary Me group (3H, 1·60 ppm, singlet) is evident. Two signals at 2·90 ppm (1H, singlet) and 5·80 ppm (1H, broad), which disappeared on addition of deuterium oxide were assigned to two OH groups. A signal at 4·02 ppm (2H, AB-type, quartet, J = 7·0 c/s) is due to hydrogens on carbon(s) bearing oxygen atom(s). For this signal two groupings, —CH_2—O— and —O—CH—CH—O— were conceivable. Though the coupling constant, 7·0 c/s, is rather small for the former, it is concluded that the grouping —CH_2—O— is the actual one, because this signal appeared as a sharp singlet in noranisatin (II) and its monoacetate IV. Further, it was a quartet with a coupling constant of 13·5 c/s, a normal value for a grouping of this type, in methyl noranisatinate acetate (X). Signals between 2·4–3·1 ppm (2H, multiplet) and at 4·66 ppm (1H, quartet) constitute a typical ABX pattern

(J_{AB} = 13·0, J_{AX} = 0·8, J_{BX} = 5·3 c/s) and are due to a —CH_2—CH(|)—O— group. This was confirmed by a spin-decoupling experiment (Fig. 1)* with noranisatin (II). A signal appearing at 4·65 ppm (1H, doublet, J = 11·0 c/s) with a concommitant decrease in the integral (corresponding to 2H) in the signals between 2·0–3·0 ppm of noranisatin (II) was attributed to a hydrogen on carbon bearing a bromine. Thus, fourteen of the fifteen hydrogens of bromonoranisatinone (VIII) could be assigned and characterized. Since the signal at 4·65 ppm was a doublet, it showed that a hydrogen was present on a carbon adjacent to the bromine-bearing carbon. Further, this hydrogen, last one to be analyzed must be attached to the carbon carrying a secondary Me group. None of the assigned hydrogens is able to couple to the secondary Me group; therefore the remaining hydrogen must be attached to the carbon bearing the secondary Me group. Since it couples to the secondary Me and to the hydrogen on carbon bearing a bromine as well, the signal appears as expected in the 2 ppm region as a multiplet.†

On the basis of the above NMR spectral analysis, partial structure of the bromoketone is shown as VIIIa. Consequently, noranisatin is represented by the partial structure IIc.



VIIIa



IIc

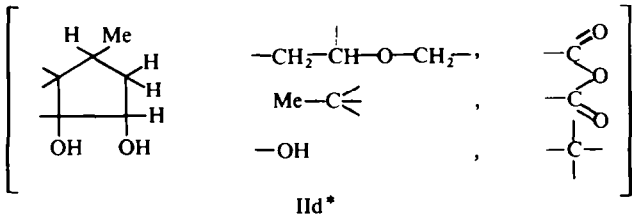
Noranisatin can now be represented by IId or IIe from the NMR analysis of the bromoketone VIII, the positions of two carbonyl bands (1832, 1778 cm^{-1}) and the fact that noranisatin consumed two moles of alkali.

(c) *Carbon skeleton.* On treatment with hydriodic acid under reflux for 20 hr, noranisatin ketoacid (VII) afforded a colorless liquid (XI) $C_{12}H_{14}O_2$. The product exhibited absorption maxima at 265 mμ (ε 550) and 275 mμ (shoulder) (ε 400) in neutral ethanolic solution, which showed bathochromic shifts (281 and 294 mμ) in an alkaline solution. Characteristic bands were observed at 3025 (ν_{C-H}, aromatic), 1775 ($\nu_{C=O}$, lactone), 1620 ($\nu_{C=C}$, aromatic), 1580 ($\nu_{C=C}$, aromatic), 810 (δ_{C-H}, two adjacent hydrogens on an aromatic ring) cm^{-1} in the IR spectrum. The NMR spectrum (carbon tetrachloride) showed signals at 1·28 ppm (3H, doublet, CH_3—CH<), 2·20 ppm (3H, singlet, aromatic Me), 2·26 ppm (3H, singlet, aromatic Me) and 6·83 ppm (2H, singlet, two aromatic protons).

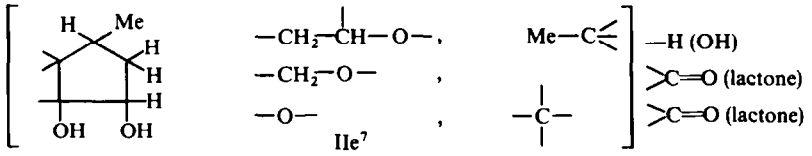
The spectral evidence indicates the presence of a secondary Me group and a benzene ring to which two Me groups are attached. From these results together with

* In the NMR spectrum (Fig. 1) of noranisatin (II), signals between 2·0–3·0 ppm (2H) and at 4·31 ppm (1H, doublet) constitute an ABX pattern (J_{AB} = 13·5, J_{AX} = 0, J_{BX} = 5·0 c/s). Irradiation of the doublet at 4·31 ppm (J = 5·0 c/s) changed the pattern in the 2·1 ppm and 2·32 ppm regions, as shown in the upper part of Fig. 1. Irradiation of the proton at 2·32 ppm collapsed the doublet at 4·31 ppm into a singlet.

† This view was verified by a spin-decoupling experiment on II. Irradiation around 2 ppm caused the doublet at 1·03 ppm of the secondary Me group (Fig. 1) to collapse to a singlet.

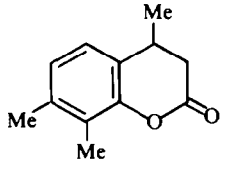


IId*

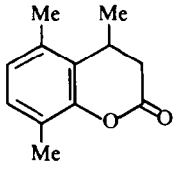


IIe[7]

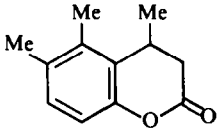
the partial structure IIc of noranisatin, the product XI is one of the three phenol lactones XIa, XIb and XIc.



XIa



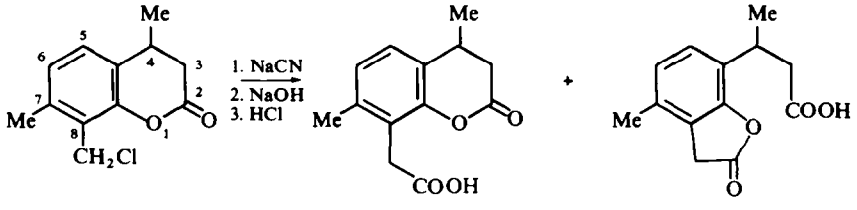
XIb



XIc

It was proved by synthesis that the product XI is the phenol lactone XIa; condensation of ethyl acetoacetate with 2,3-dimethylxylenol in sulfuric acid afforded 4,7,8-trimethylcoumarin, which was catalytically reduced with platinum in acetic acid to give 4,7,8-trimethyldihydrocoumarin. The product XI was completely identical with the synthetic specimen by spectral (IR, UV, NMR and mass) and VPC comparison. Two Me groups are present in noranisatin ketoacid (VII) and three such groups in the compound XI; one of the three Me groups in the latter was produced during the reaction with hydriodic acid.

When noranisatin ketoacid (VII) was heated in a sealed tube with acetic acid saturated with hydrogen chloride, there was obtained a dihydrocoumarin derivative (XII) $C_{12}H_{13}O_2Cl$ with spectral properties similar to those of the aforementioned

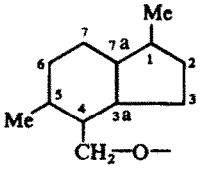


XII XIII XIV

* The possibility that a tertiary Me group is attached at one of the carbons forming a 5-membered ring must be considered but is excluded from the information regarding the carbon skeleton of noranisatin (II), which will be described subsequently.

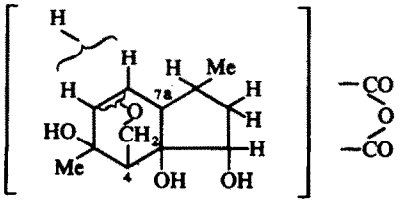
dihydrocoumarin (XI). Catalytic hydrogenation of XII afforded XI. It was deduced that a chloromethyl group is present in XII and corresponds to either of two Me groups on the benzene ring in XI. Information was obtained of the location of the chloromethyl group from the following experiments.

Reaction of XII with sodium cyanide gave a nitrile, which was hydrolyzed with aqueous sodium hydroxide. The product was heated with diluted hydrochloric acid to give a crystalline mixture of two isomeric lactone carboxylic acids XIII, XIV, which showed bands at 1805 (phenol γ-lactone), 1775 (phenol δ-lactone), 1710 (carboxylic acids) cm^{-1} in the IR spectrum. This result shows that the chloromethyl group is attached to the C-8 position of XII. On the basis of the formation of two dihydrocoumarins XI, XII from the ketoacid VII, the carbon skeleton of noranisatin is represented as IIf, which contains twelve of the fourteen carbons of noranisatin.

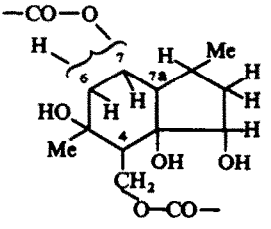


IIf

(d) *Structure of noranisatin.* In noranisatin, two of the three OH groups constitute a 1,2-glycol, while the properties of the third OH group remains unknown. From the following evidence, the OH group is tertiary; the OH in noranisatin carbonate (V) was not acetylated with acetic anhydride–pyridine at reflux or with acetyl chloride–pyridine at 90° and was resistant to oxidation with chromic acid or permanganate under a variety of conditions. The location of this tertiary OH group in the carbon skeleton IIf is deduced as follows: the ketoacid VII containing the tertiary OH group is stable to periodic acid in aqueous methanol and lead tetraacetate in acetic acid, excluding the possibility that the OH is on the C-4 or C-7a position in IIf. Since the group, —CH_2—CH—O— present in noranisatin occupies C(6)–C(7) in IIf, the only available position for the tertiary OH is C-5 which carries a tertiary Me group. From the above arguments and the carbon skeleton IIf together with the formulations IId, IIe, noranisatin can be represented as IIg or IIh. The possibility IIg must be excluded since: (i) in this case two carbons of the anhydride group are necessarily combined with C-4 and C-7a of the skeleton IIf and this is sterically impossible, (ii) hydrolysis of noranisatin monoacetate (IV) gave a monobasic acid,



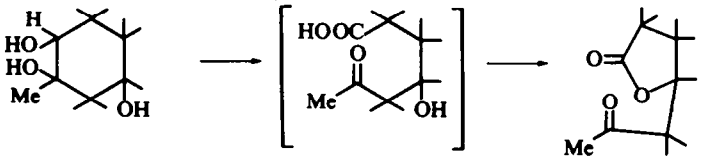
IIg



IIh

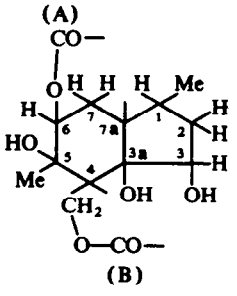
noranisatinic acid acetate (IX) with a molecular formula $C_{16}H_{22}O_9$ increased by H_2O and this finding is inconsistent with the anhydride structure, IIg. Therefore, noranisatin (II) is represented by a dilactone structure IIh.

Evidence was provided for derermining the location of the ethereal oxygen of one of the two lactones to be C-6 in IIh. Methylation of the acid IX with diazomethane afforded methyl noranisatinate acetate (X) $C_{17}H_{24}O_9$, which lacked two lactone carbonyl bands (1826, 1780 cm^{-1}) originally present in noranisatin acetate (IV), instead exhibited a strong, wide absorption at 1730 cm^{-1} (chloroform) due to superimposed carbonyl bands of the methyl ester, the acetoxyl and a newly formed δ-lactone. The methyl ester, X consumed one mole of lead tetraacetate, suggesting the formation of a 1,2-glycol upon hydrolysis of the monoacetate IV, which is inert to the same oxidizing reagent. Furthermore, oxidation of the methyl ester X with chromic acid–acetic acid or chromium trioxide–pyridine gave a neutral compound without OH groups, a ketolactone (XV), $C_{17}H_{20}O_9$, which showed bands at 1785 (γ-lactone), 1751 (δ-lactone), 1731 (methyl ester and acetate) and 1706 (ketone) cm^{-1} (KBr). The oxidation result again shows the presence of a 1,2-glycol on the six-membered ring of the methyl ester, X; the transformation with an oxidative fission of a carbon–carbon bond is illustrated by partial structures Xa, XVa. With the

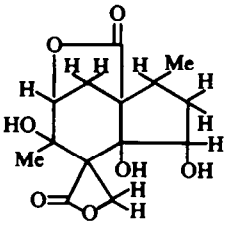


Xa XVa

information regarding the location of the lactone ether oxygen at hand, noranisatin is represented as IIi. Three conceivable structures are possible for IIi: (i) C-4 bound to C-7a to form a tricyclic structure, (ii) carbonyl A bound to C-4 and carbonyl B bound to C-7a, (iii) carbonyl A bound to C-7a and carbonyl B bound to C-4. The first structure, (i), contains a cyclopropane ring, which would be a cyclopropanone in the ketoacid, VII. A band due to a ketone carbonyl in VII appeared at 1715 cm^{-1},



IIi



IIj

excluding that possibility.* Nonexistence of a compound derived from bondings of

* The carbonyl band of a cyclopropanone should appear at a frequency higher than 1780 cm^{-1} which is the position of a carbonyl band of a cyclobutanone.[5]

the second type (ii) is apparent on steric ground. The only possible structure for noranisatin is the third one, (iii), which accounts for all the chemical and spectral features. The structure of noranisatin is thus represented by IIj.

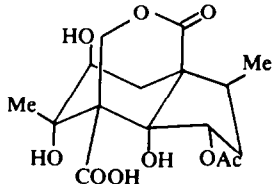
(2) *Stereochemistry of noranisatin* (II)

Formation of a 5-membered ring carbonate (V) indicates the *cis*-relationship of the 1,2-glycol in II.

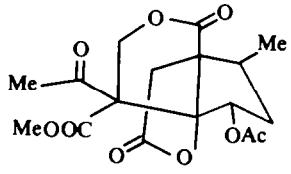
Upon hydrolysis of noranisatin monoacetate (IV), noranisatinic acid acetate (IX) was formed, which lacked the original two lactones (1826, 1780 cm^{-1}) but contained a new δ-lactone (ca. 1730 cm^{-1}), as described above. The finding clearly indicates that both β- and γ-lactones were involved for the formation of the δ-lactone. This fact together with the presence of a 1,2-glycol on a 6-membered ring shows the 1,3-diaxial relationship between the methylene group of the β-lactone and the carbonyl group of the γ-lactone in noranisatin. The stereostructure of noranisatinic acid acetate is indicated as IXa.

The rate of consumption of lead tetraacetate by methyl noranisatinate acetate (X) was slow, suggesting *trans*-relation of the 1,2-glycol on a 6-membered ring,* as indicated in the stereoformula, IXa.

Stereochemistry of the junction of two carbocyclic rings depends on the structure of the ketolactone (XV). By consideration of the structure of noranisatinic acid acetate (IX), coupled with the oxidation process already mentioned (Xa and XVa), the structure of the ketolactone is shown as XVb,† indicating unequivocally the *trans*-fusion of the rings.



IXa



XVb

The configuration of the secondary Me group remained unknown, which was firmly established as indicated in IXa and XVb by X-ray analysis[1b] of the bromonoranisatinone (VIII).

IIk

* This configurational assignment was confirmed by X-ray analysis[1b] performed on bromonoranisatinone (VIII).

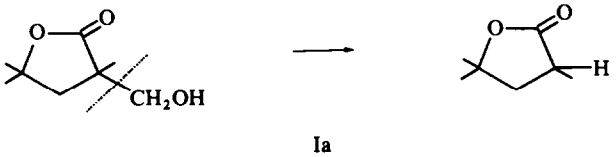
† In the mass spectrum of the ketolactone, an intense peak appeared at 326 (M^+-42), which is consistent with the substituted acetoacetate structure.[6]

From the evidence described above, noranisatin is represented as IIk. With the full knowledge of the stereochemistry of noranisatin (II), it is well understood that II lacks the reactivities typical of a β-lactone; displacement reactions from the backside at the methylene of the β-lactone are sterically inhibited; simple hydrolysis of the β-lactones causes severe steric compression.

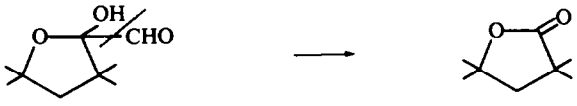
(3) *Structure of anisatin*

The structure of anisatin (I) was deduced by comparison of chemical and spectral properties between anisatin and noranisatin. Described below are important properties of anisatin (I).

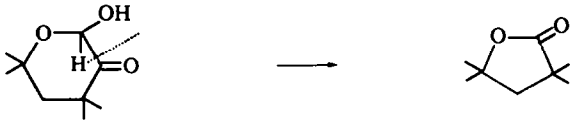
The IR spectrum of anisatin showed a broad and strong absorption band due to OH groups around 3300 cm^{-1} and two bands (1826, 1739 cm^{-1}, in chloroform) in the carbonyl region. In the UV spectrum, no appreciable absorptions were observed. Anisatin formed a monoacetate (XVI) $C_{17}H_{22}O_9$ and a triacetate* (XVII) $C_{21}H_{26}O_{11}$ under conditions specified in the experimental section. On treatment with benzoyl chloride–pyridine, anisatin gave a monobenzoate (XVIII) $C_{22}H_{24}O_9$. Similarly, anisatin monotosylate (XIX) $C_{22}H_{26}O_{10}S$ was prepared with tosyl chloride–pyridine. Anisatin gave a cyclic carbonate (XX) with phosgene in tetrahydrofuran and pyridine. One mole of periodic acid and lead tetraacetate was consumed, respectively. It was noted above that potassium permanganate oxidation of anisatin produced two neutral compounds, noranisatin (II) and noranisatinone (III). It is



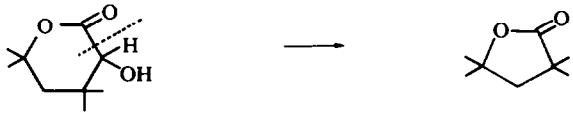
Ia



Ib



Ic



Id

* Formation of a diacetate and a triacetate from anisatin was recorded in Lane's paper.[4] The former, however, was not obtained in the present study.

evident that the carbon skeleton is intact during the oxidation of anisatin to noranisatin from the following reactions: anisatin carbonate (XX), on oxidation with potassium permanganate afforded a product which is identical with noranisatin carbonate (V), prepared from noranisatin (II).

Significant differences between anisatin (I) and noranisatin (II) are as follows: (i) the molecular formula was decreased by CH_2O on oxidation; (ii) of the two carbonyl bands (1826, 1739 cm^{-1} in I), a band at 1826 cm^{-1} remained essentially

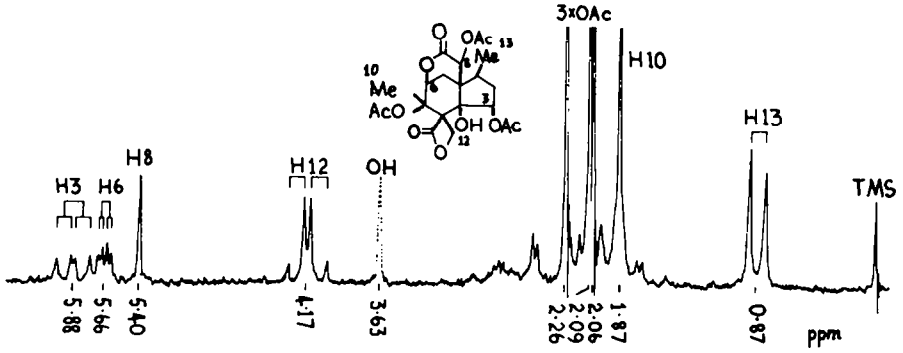
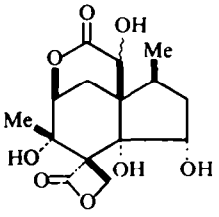


FIG. 3. The NMR spectrum of anisatin triacetate (XVII): ppm from internal TMS at 60 Mc in deuteriochloroform.

unchanged after oxidation (1832 cm^{-1} in II), whereas the second carbonyl band at 1739 cm^{-1} shifted to 1778 cm^{-1} (γ-lactone) in II; (iii) the four OH groups in I were indicated by the NMR spectrum (Fig. 3) of anisatin triacetate (XVII), while noranisatin (II) showed three. These results present the above four possibilities for the oxidation. There is no hydrogen atom alpha to the γ-lactone of II, excluding the formulation Ia. The second possibility, Ib is not the actual case, because anisatin (I) lacks aldehydic properties and an aldehydic proton was not observed in the NMR spectra of anisatin and its derivatives. Since only a negative plain curve was observed in the RD measurement of anisatin, the possibility Ic is excluded.* The fourth one must be a transformation of anisatin (I) to noranisatin (II). Evidence to support the possibility Id was provided by the NMR spectrum of anisatin triacetate (XVII) (Fig. 3). The NMR spectrum showed signals characteristic of a secondary Me group (3H, 0·87 ppm, doublet, J = 7·0 c/s), a tertiary Me group (3H, 1·87 ppm, singlet) and three acetate Me groups (2·06, 2·09, 2·26 ppm, singlet, respectively). A signal at 3·63 ppm (1H, singlet) is due to a OH hydrogen, because of the disappearance on addition of deuterium oxide; four OH groups are therefore present in anisatin (I). A quartet at 4·17 ppm (2H, J_{AB} = 7·0 c/s) could be assigned to a methylene group of the type —CH_2—O—CO—. A signal at 5·66 ppm (1H, quartet, J_{AX} = 2·0 c/s, J_{BX} = 4·0 c/s) corresponds to that of a hydrogen on the carbon carrying a γ-lactone oxygen of noranisatin (II). A signal at 5·88 ppm (1H, quartet, J_{AX} = 6·5 c/s, J_{BX} = 8·0 c/s) arises from a hydrogen on the carbon bearing a secondary OH group of a 1,2-glycol. The signals of anisatin triacetate (XVII) corresponds well to those of noranisatin (II; Fig. 1) except a signal at 5·40 ppm (1H, singlet) in the former. This signal reveals

* Formation of anhydroanisatin (XXI) without isomerization clearly excludes the possibility Ic. Details will be discussed in a section of stereochemistry of anisatin.

the presence of a hydrogen of the type —CH—O—. The presence of a secondary OH group —CH—OH is thus indicated from the above finding in conjunction with the fact that anisatin (I) has one more OH group than noranisatin (II). The deduced structure (Id) satisfactorily accounts for the observed shift of the carbonyl band and the loss of the elements CH_2O by oxidation of I to II. Anisatin is therefore represented as Ie.



Ie

(4) *Stereochemistry of anisatin*

The configuration of the secondary OH group alpha to a δ-lactone in Ie was unambiguously established by the following reactions. Since anisatin tosylate (XIX) consumed neither periodic acid nor lead tetraacetate, the secondary OH group of a 1,2-glycol was tosylated. On refluxing in pyridine, the tosylate XIX afforded anhydroanisatin (XXI) $C_{15}H_{18}O_7$ in good yield, which showed bands at 1823 (β-lactone), 1760 (shoulder, δ-lactone) and 1749 (δ-lactone) cm^{-1} (chloroform) in the IR spectrum and was transparent in the UV region. In the NMR spectrum (Fig. 4), characteristic signals appeared at: 1·04 ppm (3H, doublet, J = 7·0 c/s), 1·54 ppm (3H, singlet), 4·04 ppm (1H, multiplet), 4·25 ppm (1H, singlet), 4·32 ppm (2H, quartet, AB-type, J_{AB} = 7·0 c/s), 4·37 ppm (1H, quartet, J_{AX} = 2·0 c/s, J_{BX} = 4·0 c/s), 4·40 ppm (1H, singlet, OH) and 5·34 ppm (1H, singlet, OH). The NMR spectral result reveals that two of the four OH groups of anisatin (I) are present in anhydroanisatin (XXI) and further that the three hydrogens (4·04, 4·25 and 4·37 ppm, respectively) of the type —CH—O— in I are retained in anhydroanisatin (XXI). Other spectral features of

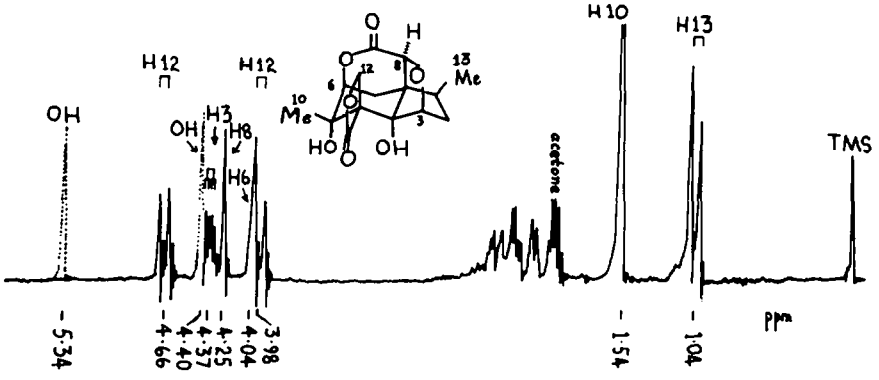
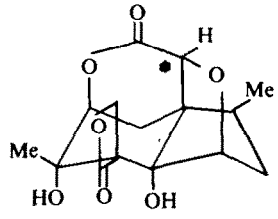


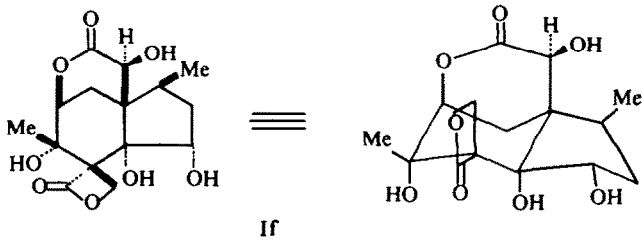
FIG. 4. The NMR spectrum of anhydroanisatin (XXI): ppm from internal TMS at 100 Mc in deuterioacetone.

anhydroanisatin were well correlated with those of anisatin. From the NMR spectral evidence, it was deduced that dehydration took place between two OH groups of anisatin to form an ether linkage. Evidently two secondary OH groups of I were concerned with the formation of the ether bond, because of the inertness of anhydroanisatin (XXI) to a variety of oxidations on which anisatin (I) gave II and III. The structure of anhydroanisatin is thus established as XXIa with a tetrahydrofuran ring.



XXIa

Formation of anhydroanisatin (XXI) indicates that the secondary OH group is so oriented as to cause the intramolecular displacement of a tosyloxy group. Assuming that epimerization did not occur at the carbon atom alpha to a δ-lactone during the formation of XXI, the configuration of the secondary OH group in question is as shown in If. The assumption was verified by the following results. If the configuration of the secondary OH group is opposite to that shown in If, epimerization must occur via an enol form of the δ-lactone before the formation of a tetrahydrofuran ring. The tosylate XIX was thus heated in pyridine containing deuterium oxide (5%) under the same condition as described above to afford a product, which was washed thoroughly with water. Examination of the resulting anhydroanisatin (XXI) by NMR and mass spectral methods showed that the deuteration of the hydrogen on the



If

asterisked carbon in XXIa took place to a minor extent (i.e. less than 25%). The possibility is excluded that the conversion of the tosylate (XIX) to XXI proceeds with inversion of the configuration. The configuration of the secondary OH group is therefore established as If on the basis of the formation of XXI.

With all the evidence described above, the complete stereostructure of anisatin is established as If.

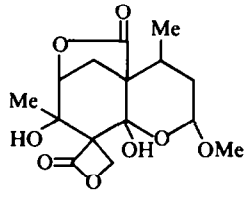
(5) *Other reactions of anisatin*

During the structural studies of anisatin (I), a number of reactions was carried out; some of them will be described and discussed.

(a) *Oxidation.* Vigorous oxidation of anisatin with chromic acid in 6N H_2SO_4

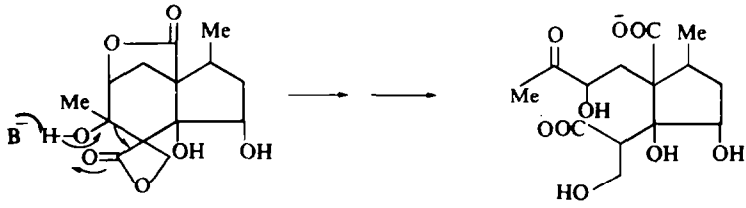
gave methylsuccinic acid in addition to noranisatinone (III) and the ketoacid (VII). Methylsuccinic acid must arise from the carbocyclic 5-membered ring carrying a secondary Me group.

Noranisatin (II), on oxidation with lead tetraacetate afforded the ketoaldehyde VI (*vide supra*), whereas with periodic acid in methanol it gave a compound $C_{14}H_{16}O_7 \cdot CH_3OH$ (XXII), the IR spectrum of which showed bands at 1825 (β-lactone) and 1786 (γ-lactone) cm^{-1}. The absence of a ketonic and an aldehydic carbonyl band must be concerned with the increased molecular formula by the elements of methanol. An acetal-hemiketal structure, XXIIa, was assigned to the compound.



XXIIa

The iodoform reaction was positive with noranisatin (II), while negative with anisatin (I). The positive result is presumably accounted for by a retroaldol reaction between the tertiary hydroxyl group and the β-lactone (or more probably, the carboxylate), as indicated below. The failure of the iodoform reaction with anisatin (I) is not well reationalized, but might be ascribed to the more rapid isomerization of anisatin (I) to anisatinic acid[4] with alkali, which gave a negative iodoform test. The isomerization reaction of I to anisatinic acid is complex and will be described elsewhere.



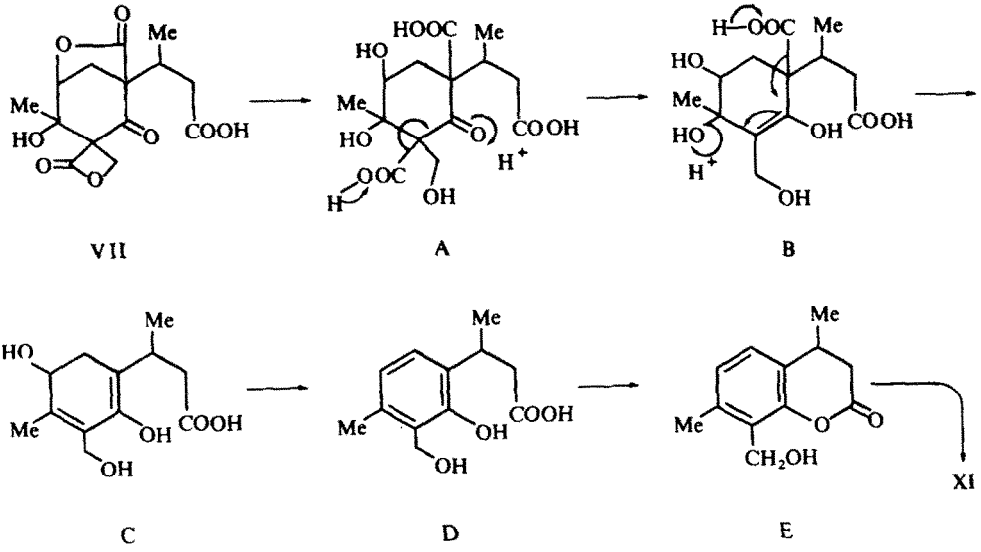
II

(b) *Action of acids.* Anisatin is quite stable to various acids (HCl, HBr, HI, conc H_2SO_4, H_3PO_4, p-TsOH, HNO_3 and BF_3-etherate) even at elevated temperatures; neither dehydration nor hydrolysis occurred presumably owing to the rigidity of the anisatin molecule. Noranisatin (II) behaves similarly to acids; however, noranisatin ketoacid (VII), in contrast to I and II, might be flexible enough to assume a variety of conformations and probably for this reason reacted with acid to afford dihydrocoumarin (XI), formation of which can be explained as follows.* Acidic hydrolysis of the β-lactone (or possibly both lactone groups) in the ketoacid VII took place to some extent, giving an intermediate A. The newly formed carboxylic acid would decarboxylate easily to afford B, since a ketone is located at the β-position of the carboxyl group. The next step would be a dehydration of a tertiary OH group

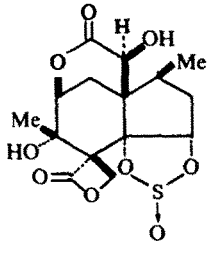
* The explanation described here, is presented merely as one of the several possibilities.

with simultaneous decarboxylation to give C, which in turn with loss of one mole of water would be transformed to a phenolic carboxylic acid (D). Lactonization of D led to an intermediate E, which on reduction of the hydroxymethyl group by hydriodic acid gave the dihydrocoumarin XI.

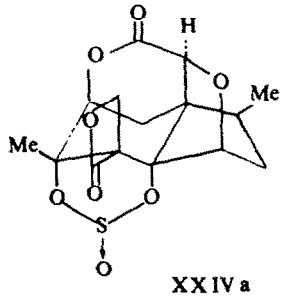
Scheme II



For the purpose of dehydration of anisatin (I), phosphorus oxychloride was employed to give unaltered anisatin. On the other hand, treatment of anisatin with thionyl chloride and pyridine afforded a cyclic sulfite (XXIII) $C_{15}H_{18}O_9S$, the structure



XXIIIa



XXIVa

of which is indicated as XXIIIa by analogy with the carbonate XX. Further, anhydroanisatin sulfite (XXIV) $C_{15}H_{16}O_8S$ was prepared from the sulfite XXIII or directly from anisatin under the condition of longer reaction time and higher temperature. Anhydrosulfite XXIV contains none of the hydroxyl groups and was assumed to have a structure XXIVa with a tetrahydrofuran ring. The anhydrosulfite was unstable and gradually turned to an oil.

NEOANISATIN

(6) *Properties of neoanisatin*

Separation of neoanisatin (XXV) from anisatin (I) was achieved by column chromatography on alumina. The molecular formula $C_{15}H_{20}O_7$ was determined by elemental analysis and mass spectrometry (M^+312). Neoanisatin formed a diacetate (XXVI) $C_{19}H_{24}O_9$ with acetic anhydride and pyridine. Close similarity of anisatin and neoanisatin was indicated by spectral data: the IR spectrum of neoanisatin showed two carbonyl bands (1823, 1733 cm^{-1}, chloroform) and other IR spectral

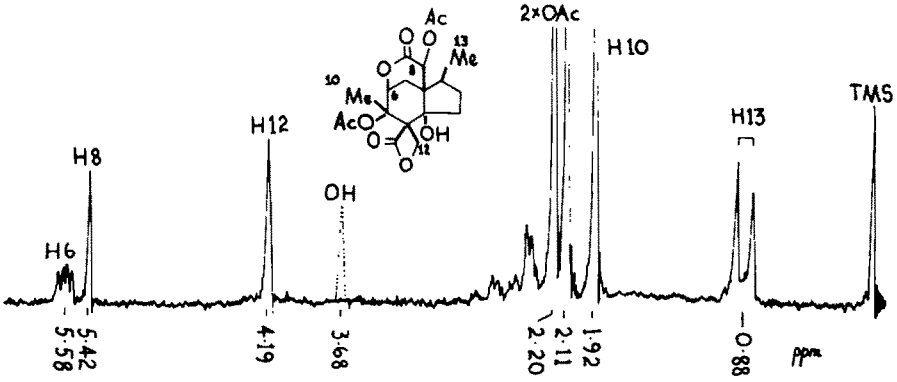


FIG. 5. The NMR spectrum of neoanisatin diacetate (XXVI): ppm from internal TMS at 60 Mc in deuteriochloroform.

features were similar to those of anisatin. Resemblance of the NMR patterns between two compounds was also noted (cf. Fig. 3 and 5). In the NMR spectrum (Fig. 5) of neoanisatin diacetate (XXVI), a signal at 3·68 ppm (1H, singlet) was assigned to a OH group, which disappeared on addition of deuterium oxide. Therefore, neoanisatin (XXV) has three OH groups.

As described above, anisatin (I) gave a monobenzoate (XVIII) and a carbonate (XX), whereas corresponding derivatives were not obtained from neoanisatin (XXV) under the same conditions.

These findings suggest that neoanisatin (XXV) has the same structure as anisatin (I) except for the absence of the secondary hydroxyl group of the 1,2-glycol in I. This view was substantiated by a series of reactions described later. Correlation of two toxic compounds, I, XXV was carried out by obtaining a common derivative, the ketoacid VII.

(7) *Structure of norneoanisatin*

A parallel result between anisatin (I) and neoanisatin (XXV) was obtained on oxidation with potassium permanganate. The product, norneoanisatin (XXVII) $C_{14}H_{18}O_6$ showed carbonyl bands at 1832 and 1776 cm^{-1} (chloroform). In the NMR spectrum, there were observed prominent signals arising from a secondary Me (3H, 1·01 ppm, doublet, J = 6·5 c/s), a tertiary Me (3H, 1·55 ppm, singlet), a methylene of the type —CH_2—O— (2H, 4·15 ppm, singlet), a methine of the type —CH(OH)—O— (1H, 4·28 ppm, doublet, J = 5·0 c/s) and two OH groups (detected by hydrogen–deuterium exchange on addition of deuterium oxide). The spectral (IR and NMR)

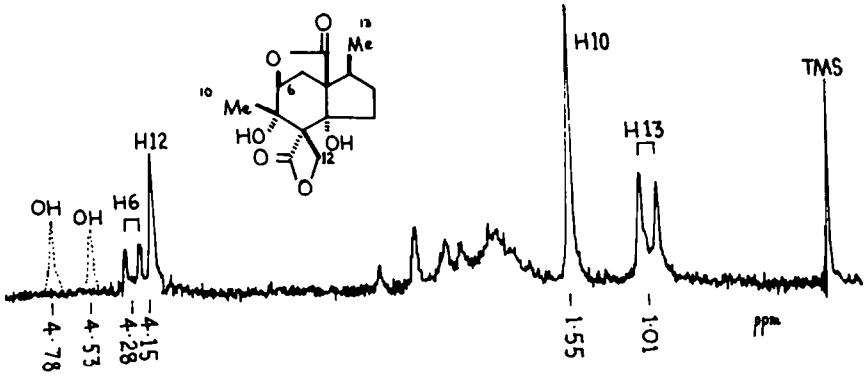
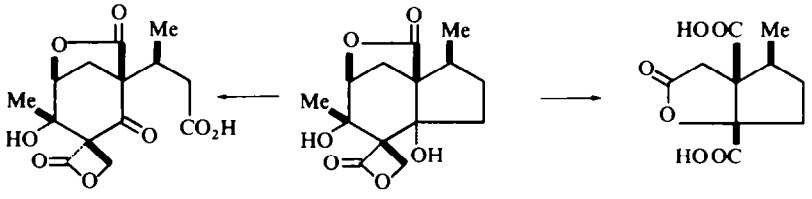


FIG. 6. The NMR spectrum of norneoanisatin (XXVII): ppm from internal TMS at 60 Mc in deuteriochloroform.

similarity between nor-derivatives, II, XXVII of anisatin (I) and neoanisatin (XXV) was again noted.

Norneoanisatin (XXVII) was recovered unchanged under mild oxidation conditions: chromic acid in aqueous acetic acid at room temperature and potassium permanganate in aqueous acetic acid. Oxidation of norneoanisatin (XXVII) did occur, however, with chromium trioxide in acetic acid at 60–70°, to afford a ketoacid in good yield without the loss of carbon. The ketoacid, thus obtained turned out to



VII XXVIIa XXVIIIa

be identical with the one (VII) prepared from anisatin (I). Since a 1,2-glycol is absent in norneoanisatin (XXVII), the above findings permitted the assignment of the structure, XXVIIa to norneoanisatin, in which the configuration of the angular OH group is unsettled.

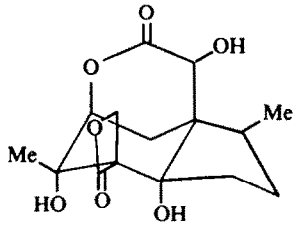
Evidence is presented as regards the stereochemistry of the angular hydroxyl group from the following oxidation result: chromium trioxide oxidation of norneoanisatin (XXVII) in aqueous sulfuric acid at 80–85° afforded a lactonic diacid (XXVIII) $C_{10}H_{12}O_6$ (pKa' in water, 3·6 and 4·8). The product XXVIII showed bands at 1803 and 1700 cm^{-1} (KBr) in the IR spectrum. In the sodium salt, no carbonyl bands appeared at frequencies higher than 1650 cm^{-1}. Instead a broad, strong band was observed around 1600 cm^{-1} (carboxylates). On acidification of the sodium salt, the lactone diacid was again obtained with regeneration of the band at 1803 cm^{-1}. These observations clearly reveal the presence of a γ-lactone ($\nu_{C=O}$, 1803 cm^{-1}) in XXVIII. The NMR spectrum of XXVIII in deuterioacetone showed a doublet at 1·08 ppm (3H, J = 6·5 c/s) arising from a secondary Me and multiplets around 2·2 ppm (5H, measured in pyridine) due to methylene and methine protones.

There were observed an AB-type signal centered at 2·86 ppm (2H, J_{AB} = 18 c/s) and a singlet at 9·3 ppm (2H), the former being assigned to an additional methylene group and the latter to carboxyl groups. From these findings together with the structure of norneoanisatin (XXVII), the lactone diacid can be formulated as XXVIIIa. Two carboxyl groups of XXVIII formed a 5-membered ring anhydride ($\nu_{C=O}$, 1865, 1800 cm^{-1}) on treatment with acetic anhydride. Formation of the anhydride and the presence of a γ-lactone ring indicate the *cis* relationship between the angular OH group and the methylene of the 6-membered ring in norneoanisatin (XXVII), thus the carbocyclic rings are *trans* fused.

(8) *Structure of neoanisatin*

The conversion of neoanisatin (XXV) to norneoanisatin (XXVII) parallels that of anisatin (I) to noranisatin (II). Of the two carbonyl bands (1823, 1733 cm^{-1}) of neoanisatin (XXV), a higher frequency shift of the second band was observed after oxidation; the nor-derivative XXVII showed the band at 1776 cm^{-1} (γ-lactone). The elements CH_2O were lost by the oxidation, which is certainly associated with the γ-lactone moiety of the nor-derivative XXVII.

Neoanisatin (XXV) has three OH groups, whereas two are present in norneoanisatin



XXVa

(XXVII). The OH group lost during oxidation is secondary, because XXVII has no signal corresponding to the one at 5·42 ppm (1H, singlet) of the type —CH(|)—O— found in the NMR spectrum (Fig. 5) of neoanisatin diacetate (XXVI). These observations led to a conclusion that an α-hydroxy(secondary)-δ-lactone was converted to a γ-lactone during the oxidation. Neoanisatin is therefore represented as XXVa.

(9) *Other reactions of norneoanisatin* (XXVII)

A variety of reactions was examined on norneoanisatin (XXVII) in connection with the structure proof. Some results are recorded below and interpreted by virtue of chemical and spectral data.

(a) *Hydrolysis of norneoanisatin* (XXVII). Treatment of XXVII with aqueous barium hydroxide afforded a lactonic carboxylic acid, norneoanisatinic acid (XXIX) $C_{14}H_{20}O_7$ ($\nu_{C=O}$, 1723, 1704 cm^{-1}) and an amorphous compound. With diazomethane, the acid XXIX was converted to methyl norneoanisatinate (XXX) $C_{15}H_{22}O_7$ ($\nu_{C=O}$, 1738 cm^{-1}), which consumed one mole of lead tetraacetate. The NMR spectrum of the ester and assignments of the characteristic signals are shown in Fig. 7.

Chromic acid oxidation of the methyl ester (XXX) gave a neoketolactone (XXXI) $C_{15}H_{18}O_7$, which showed carbonyl bands at 1775, 1735, 1725 and 1701 cm^{-1} and

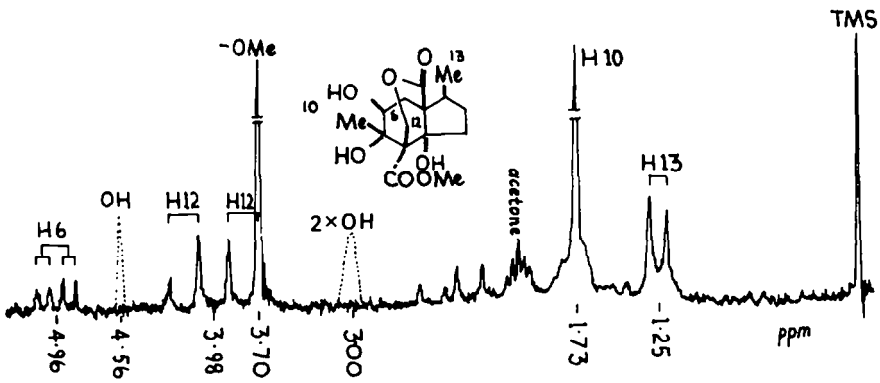
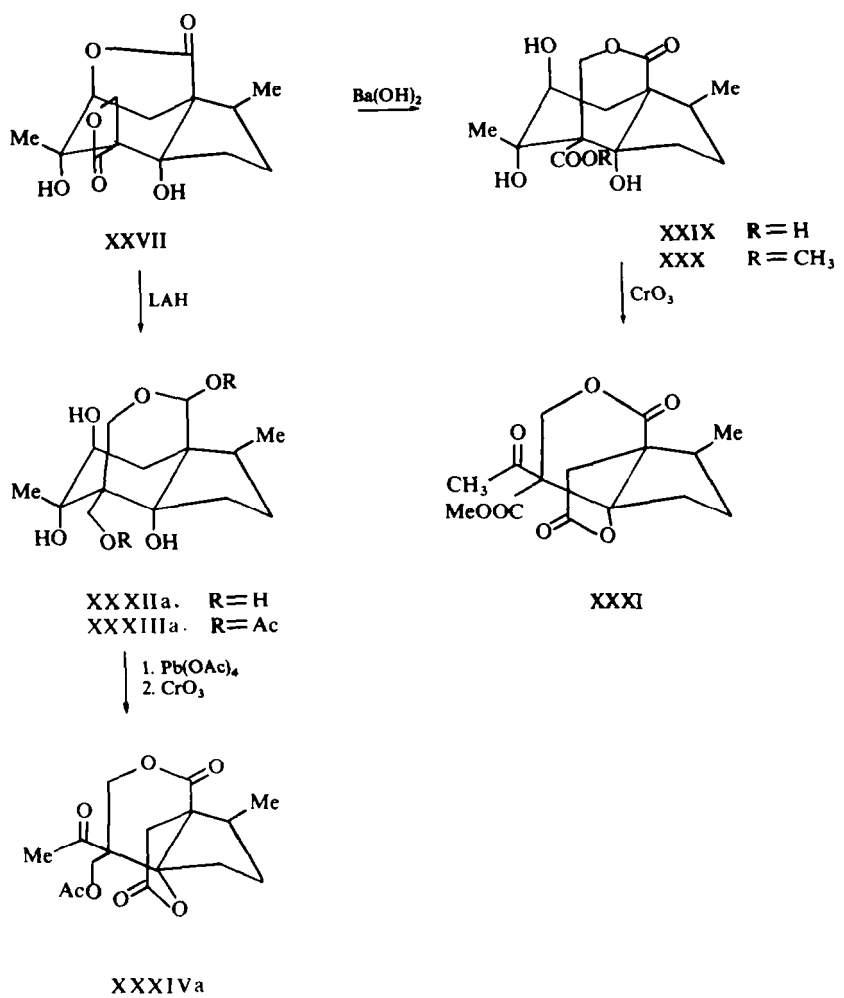


FIG. 7. The NMR spectrum of methyl norneoanisatinate (XXX): ppm from internal TMS at 60 Mc in deuterioacetone.

Scheme III. Derivatives of norneoanisatin (XXVII)



no hydroxyl bands in the 3000 cm^{-1} region. The series of reactions described above is analogous to that found in noranisatin (II) and is illustrated in Scheme III.

(b) *Lithium aluminum hydride reduction of norneoanisatin (XXVII).* Norneoanisatin (XXVII), on reduction with LAH in tetrahydrofuran gave hexahydro norneoanisatin (XXXII) $C_{14}H_{24}O_6$, which showed no carbonyl absorptions in the IR spectrum. If complete reduction of two lactones in XXVII had occurred, the product would be an octahydro derivative. Formation of the hexahydro derivative XXXII suggests that while one of the two lactone rings was converted to a diol, the reduction of the other was stopped at the stage of an aldehyde, in which intramolecular hydration occurred to afford a cyclic hemiacetal. This interpretation is consistent with the NMR spectrum (Fig. 8) of XXXII. In addition to the signal (4·51 ppm, AB-type, J_{AB} = 11·5 c/s) of the type —CH_2—O— originally present in norneoanisatin (XXVII), a signal appeared at 4·30 ppm (2H, AB-type, J_{AB} = 12·5 c/s) due to a methylene, —CH_2—O—. A multiplet at 4·12 ppm (1H) can be assigned to a methine of the type —CH—O— and corresponds to a hydrogen on carbon bearing a lactone
|
ether oxygen of norneoanisatin (XXVII). A supporting evidence of the presence of a hemiacetal is provided by a singlet at 5·25 ppm (1H). On the basis of the NMR spectrum together with the fact that the diacetate (XXXIII) of the hexahydro compound consumed one mole of lead tetraacetate, the structure XXXIIa was assigned to the reduction product (Scheme III). Either one of two plausible reasons is responsible for the formation of the hexahydro derivative XXXII instead of the expected octahydro compound; the first one is that the metal complex formed with the reactant became insoluble before complete reduction of two lactone rings; the second is that the formation of the octahydro compound would result in a severe steric compression caused by three 1,3-interactions.

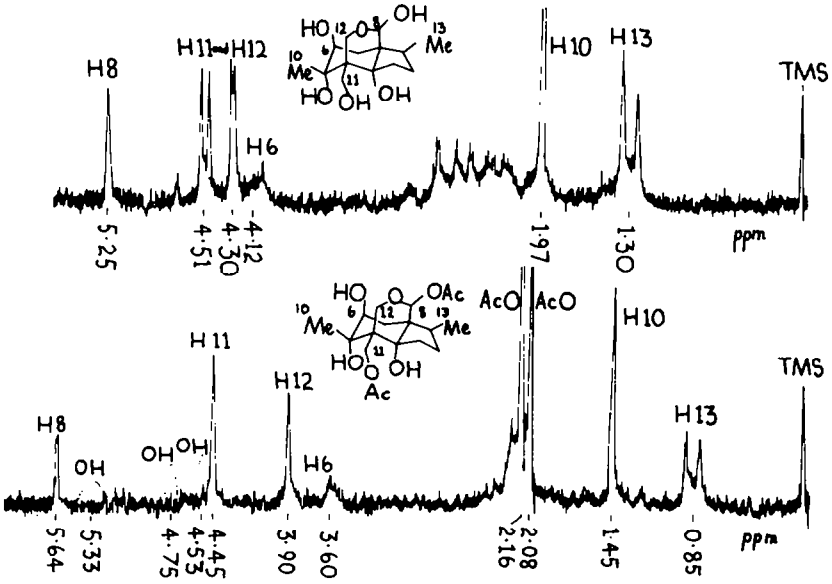


FIG. 8. The NMR spectra of hexahydro norneoanisatin (XXXII) in pyridine and of its acetate (XXXIII) in deuteriochloroform: ppm from internal TMS at 60 Mc.

On acetylation of the hexahydro compound XXXII with acetic anhydride and pyridine, hexahydro norneoanisatin diacetate (XXXIII) $C_{18}H_{28}O_8$ ($\nu_{C=O}$, 1745 cm^{-1}) was formed. Since the diacetate XXXIII consumed one mole of lead tetraacetate, the structure XXXIIIa was assigned (Scheme III), which is consistent with the NMR spectrum (Fig. 8) of the diacetate XXXIII.

Oxidation of the diacetate XXXIII with lead tetraacetate afforded an oily product, which was subsequently oxidized with chromic acid to give a neutral compound, neoketolactone monoacetate (XXXIV) $C_{16}H_{20}O_7$. The IR spectrum revealed four carbonyl bands at 1778, 1745, 1725 and 1701 cm^{-1} (KBr) and the absence of OH groups. In the NMR spectrum (Fig. 9), characteristic signals appeared arising from three methylene groups of the type —CH_2—O—, whereas a singlet due to a hydrogen —O—CH—O— of the diacetate XXXIII was absent. One of the acetoxyl groups in XXXIII was lost during oxidation, which was revealed by a single acetoxyl signal at 2·39 ppm. These observations led to a formula, XXXIVa for the oxidation product.

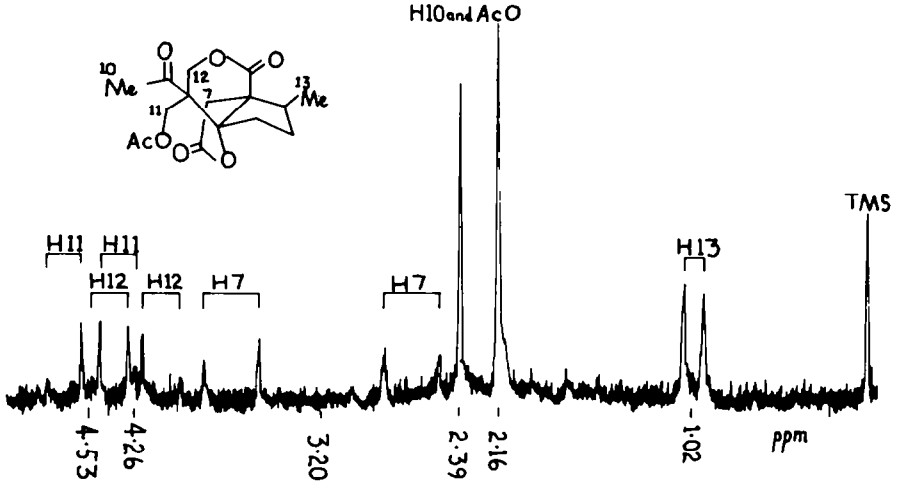


FIG. 9. The NMR spectrum of neoketolactone monoacetate (XXXIV): ppm from internal TMS at 60 Mc in deuteriochloroform.

EXPERIMENTAL

All m.ps were uncorrected. The UV spectra were measured in EtOH soln with a Beckman DK-2 Spectrophotometer. The IR spectra were recorded with a Nihon-Bunko IR-S Spectrophotometer and with a Nihon-Bunko DS-402G Spectrophotometer. Optical rotations were taken on a Rudolph Spectropolarimeter. The NMR spectra were recorded with Varian Associates spectrometers (A60 and HA-100); only prominent peaks are cited; the chemical shifts are given in ppm relative to internal TMS; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; coupling constants are given in c/s. The mass spectra were determined on a Hitachi RMU-6D mass spectrometer equipped with a direct inlet system and operating with an ionization energy of 70 eV. For VPC, a Yanagimoto model GCG-2 was employed, operating with a 1·5 m × 4 mm column packed with 5% SE-30 on Chromosorb W at 147° (He as carrier gas). TLC analysis was performed on silica gel G or silica gel GF (E. Merck, A.G., Germany). For column chromatography, Wako alumina (Wako, Japan) and Mallinckrodt silicic acid (100 mesh, Mallinckrodt, U.S.A.) were used.

Extraction of anisatin (I) *and neoanisatin* (XXV)

The seeds of *Illicium Anisatum* L. collected in October–November in Goto Islands (Kyushu district of Japan) were ground. For defatting, n-hexane was added and the mixture was left for several days with occasional stirring and was filtered with suction. This defatting procedure was repeated 3 times to give powdered material.

(a) Defatted powdered material was put in a drum, which was filled with MeOH. After several days, a red MeOH solution was collected from the cock attached to the drum. The extraction with MeOH was repeated 3 times. This procedure was convenient for large-scale extraction.

(b) Continuous extraction of the defatted powdered material (12 kg) was carried out with warm EtOH for 30 hr. The EtOH extract was dark-red. This procedure gave toxic compounds in better yield than the procedure (a).

The EtOH extract obtained from the procedure (b) was concentrated under red. press. to give a semisolid: water was added and the mixture was again concentrated in order to remove the remaining EtOH. To the dark-red residue was added AcOEt (1000 ml) and the mixture was refluxed for 10 hr with occasional stirring. After separation of the dark-red AcOEt extract by decantation, the semisolid residue was further extracted with two 1000 ml portions of AcOEt by the same procedure as described above. The combined AcOEt extracts were concentrated under red. press. to afford a brown oily material, which was washed with n-hexane repeatedly. To the resulting dark-brown semisolid was added AcOEt (500 ml) and the insoluble material was removed by filtration. The filtrate was shaken with acidic alumina (40–50 g).* The mixture was filtered and the filtrate was concentrated under red. press. to give a viscous yellow-brown soln (100 ml), from which crude crystals (2–2·5 g) of anisatin (I) precipitated within 2 days. After separation of crude I by filtration, the filtrate was further concentrated to afford a crystalline mixture of I and XXV. Crude crystals of I were purified by chromatography over acid-washed alumina using AcOEt as the eluting solvent and recrystallization from AcOEt yielded crystals of I containing 1 mole of AcOEt, m.p. 215–220°. Recrystallization from H_2O gave needles of I, m.p. 227–228°; IR bands at 1826, 1739 cm^{-1} ($CHCl_3$); UV, end absorption at 220 mμ (ε 330); mass 328 (M^+). (Found: C, 55·00; H, 6·08. $C_{15}H_{20}O_8$ requires: C, 54·87; H, 6·14%.) $[\alpha]_D^{20}$ −28° (*c* 2, dioxan).

Separation of anisatin (I) *and neoanisatin* (XXV)

(a) A mixture (1 g) of I and XXV was dissolved in AcOEt (ca. 30 ml) and chromatographed over acid-washed alumina, using AcOEt as the eluting solvent. Early fractions contained XXV and then I was obtained. Complete separation of both compounds was difficult.

(b) A mixture of I and XXV was fractionally recrystallized: if the mixture contained comparable amounts of I and XXV, I crystallized first. Both I and XXV contain 1 mole of AcOEt as solvent of crystallization respectively, which was indicated by the NMR spectra. Pure XXV was obtained by combined methods of fractional recrystallization and chromatography. Recrystallization from H_2O gave colorless needles, m.p. 237–238°; $[\alpha]_D^{25}$ −25° (*c* 1, dioxan); IR bands at 1823, 1733 cm^{-1} ($CHCl_3$); UV, end absorption at 220 mμ (ε 290); mass 312 (M^+). (Found: C, 57·77; H, 6·60. $C_{15}H_{20}O_7$ requires: C, 57·68; H, 6·46%.)

Action of HIO_4 *on* I *and* XXV

Samples (10–20 mg) were allowed to react with excess HIO_4 in AcOH–H_2O (4:1) for varying lengths of time, after which KI was added and the liberated I_2 titrated with $Na_2S_2O_3$ aq (temp 18–25°).

I: 0·10 mole (1·5 hr), 0·50 mole (25 hr), 0·87 mole (76 hr), 0·91 mole (104 hr).
XXV: 0·00 mole (1·5 hr), 0·01 mole (104 hr).

Action of $Pb(OAc)_4$ *on* I *and* XXV

Samples (20–25 mg) were allowed to react with excess $Pb(OAc)_4$ in AcOH for varying lengths of time, after which a solution of KI–AcONa was added and the liberated I_2 titrated with a standardized solution of $Na_2S_2O_3$ (temp 25–26°).

I: 0·85 mole (10 min), 0·93 mole (30 min), 0·95 mole (1 hr).
XXV: 0·01 mole (10 min), 0·01 mole (1 hr).

* Commercially available Wako Alumina was washed with dil H_2SO_4 until the washing showed pH 4·5. The acid-treated alumina was washed with distilled water, filtered and activated at 140–150° for 15 hr.

Methylsuccinic acid

A CrO_3 soln (1·3 g/14 ml) was added within 5 hr to a suspension of I (500 mg) in 6N H_2SO_4 (25 ml) heated at 80–90°. At the end of addition of the oxidizing agent, almost clear soln was obtained, which was concentrated under red. press. The concentrate was continuously extracted with ether. The extract was dried over Na_2SO_4 and concentrated to give a yellow oil (300 mg), which was again dissolved in ether (50 ml) and extracted with two 10 ml portions of sat. $NaHCO_3$aq. The combined $NaHCO_3$ layers were made acidic with 4N H_2SO_4 and extracted continuously with ether. The ethereal extract was dried over Na_2SO_4 and concentrated to give an oil (140 mg), which was chromatographed on silicic acid: elution with $CHCl_3$–AcOEt (6:1) gave a crystalline solid (40 mg), the mass spectrum of which was identical with that of methylsuccinic acid. Part of the solid was dissolved in MeOH and neutralized with $NaHCO_3$aq. Concentration of the soln gave the Na salt (50 mg), which was dissolved in H_2O (0·5 ml), mixed with a MeOH solution of *p*-bromophenacyl bromide (130 mg/9 ml) and refluxed for 2 hr. On cooling, white crystals were deposited: recrystallization from CCl_4 gave needles, m.p. 136·5–138°; the IR spectrum was superimposable with that of an authentic sample. (Found: C, 48·20; H, 3·80. $C_{21}H_{18}O_6Br_2$ requires: C, 47·93; H, 3·45%.)

Noranisatin (II) *and noranisatinone* (III)

To a soln of I (5·40 g) in AcOH (130 ml) was added $KMnO_4$aq (5·40 g/100 ml). The mixture was stirred overnight at room temp and the excess $KMnO_4$ was reduced with $NaHSO_3$aq. The clear soln was concentrated under red. press. to give a white solid, which was removed by filtration. The filtrate was diluted with H_2O and extracted with three 60 ml portions of AcOEt. The combined AcOEt extracts were washed with H_2O and sat. NaClaq and dried over Na_2SO_4. On removal of Na_2SO_4, followed by evaporation of the solvent, a yellow oily product (4·32 g) was obtained, which was chromatographed over silicic acid (80 g). Early fractions (total amount, 100 ml) eluted by $CHCl_3$–AcOEt (3:1) gave a crystalline product (0·92 g), recrystallization of which from $CHCl_3$ afforded colorless needles of III, m.p. 213–215°, IR bands at 3500, 1831, 1789, 1754 cm^{-1} ($CHCl_3$); NMR signals at 1·15 (3H, d, J = 7·0), 1·66 (3H, s), 2·43 (1H, q, J_{AB} = 12·5, J_{AX} = 5·5, A-part of an ABX pattern), 3·08 (1H, d, J_{AB} = 12·5, J_{BX} = 0, B-part of an ABX pattern), 4·60 (1H, d, J_{AX} = 5·5, J_{BX} = 0, X-part of an ABX pattern), ~2·7 (4–5H, m), 4·33 (2H, q, J = 7·0, AB-type); mass 296 (M^+). (Found: C, 56·43; H, 5·76. $C_{14}H_{16}O_7$ requires: C, 56·75; H, 5·44%.)

From the later fractions (ca. 280 ml), II (1·29 g) was obtained and recrystallized from $CHCl_3$–ether, m.p. 163–164°; IR bands at 3460, 1332, 1778 cm^{-1} ($CHCl_3$); NMR spectrum (Fig. 1). (Found: C, 56·27; H, 6·13. $C_{14}H_{18}O_7$ requires: C, 56·37; H, 6·08%.)

Titration of noranisatin (II)

A soln of II (74 mg) in 0·067N NaOH (25 ml) was kept at room temp for 6 hr under N_2. The soln (5·00 ml each) was titrated with 0·023N HCl, using phenolphthalein as an indicator; 10·18 ml of HClaq was required. Blank titration; for 5·00 ml of 0·067N NaOH, 14·10 ml of 0·023N HClaq was consumed. The data showed 1 mole of II consumed 1·88 moles of alkali.

Oxidation of noranisatin (II) *to noranisatinone* (III)

To a soln of II (250 mg) in AcOH (10 ml) was added a soln of CrO_3 (200 mg) in AcOH (1 ml). The mixture was kept at room temp for 24 hr and $NaHSO_3$aq was added. The mixture was poured into H_2O and extracted with AcOEt. The extract was washed with H_2O, sat. NaClaq and dried over Na_2SO_4. On removal of the solvent, light-greenish oily product (300 mg) was obtained and chromatographed on silicic acid (3 g) using $CHCl_3$ as the eluting solvent. Early fractions (30 ml) gave crystals (84 mg), which on recrystallization from $CHCl_3$ yielded needles, m.p. 213–215°, identified as III by the IR spectra and mixed m.p. Later fractions afforded a white solid (90 mg), which was recrystallized from $CHCl_3$–AcOEt to give needles, m.p. 152–154°, identified as the ketoacid VII by the IR spectra and mixed m.p.

Noranisatin monoacetate (IV)

A soln of II (80 mg) in pyridine (1 ml)–Ac_2O (0·2 ml) was kept at room temp for 15 hr, poured into ice-water and extracted with AcOEt. The extract was washed with H_2O, sat. NaHClaq and dried over Na_2SO_4. On evaporation of the solvent, there remained a white solid, which on recrystallization from $CHCl_3$–ether gave plates of IV, 50 mg, m.p. 186–188°; IR bands at 3400, 1820, 1770, 1725 cm^{-1} (KBr); NMR signals ($CDCl_3$) at 1·12 (3H, d, J = 6·5), 1·54 (3H, s), 2·09 (3H, s), 3·90 (1H, s, OH), 4·2 (1H, broad,

OH), 4·26 (2H, s), 4·37 (1H, d, J = 5·5), 5·67 (1H, q, J_{AX} = 7·0, J_{BX} = 9·5). (Found: C, 56·56; H, 5·80. $C_{16}H_{20}O_8$ requires: C, 56·46; H, 5·92%.)

Noranisatin carbonate (V)

Phosgene gas was passed through the soln of II (300 mg) in THF (5 ml)–pyridine (0·5 ml) cooled in an ice-bath for 45 min. A white solid was deposited and the mixture was concentrated under red. press. On addition of H_2O, the residue gradually crystallized (200 mg), which on recrystallization from $CHCl_3$–AcOEt gave needles of V, m.p. 236–238°, IR bands at 3520, 1830, 1798 (5-membered ring carbonate), 1770 cm^{-1} (KBr); NMR signals (CD_3COCD_3) at 1·13 (3H, d, J = 5·5), 1·65 (3H, s), 4·35 (2H, q, AB-type, J_{AB} = 7·5), 4·59 (1H, d, J = 5·0), 4·71 (1H, s, OH), 5·42 (1H, d, J = 5·0). (Found: C, 55·71; H, 5·00. $C_{15}H_{16}O_8$ requires: C, 55·55; H, 4·97%.)

Attempted acetylation of noranisatin carbonate (V)

(a) A soln of V (110 mg) in pyridine (2 ml)–Ac_2O (0·4 ml) was refluxed for 5 hr. On complete removal of the solvent, there remained a white solid, to which H_2O was added. The solid was filtered and recrystallized from $CHCl_3$–AcOEt to give needles, which was identified as the starting material by the IR spectra.

(b) A soln of V (135 mg) in AcCl (0·1 ml)–pyridine (0·1 ml)–THF (3 ml) was allowed to stand for 3 days and refluxed for 1 hr. The solvent was removed and and the residue was diluted with H_2O, extracted with AcOEt. The extract was washed with $CdCl_2$aq, $NaHCO_3$aq, sat. NaClaq and dried over Na_2SO_4. Concentration of the soln gave a white solid, which was recrystallized from $CHCl_3$–AcOEt to afford crystals. These proved to be starting material by comparison of IR spectra.

Attempted oxidation of noranisatin carbonate (V)

A soln of V (100 mg) in AcOH (20 ml) was mixed with a chromic acid soln (CrO_3 70 mg/H_2O 5 ml). The mixture was heated at 60–70° for 3·5 hr, after cooling diluted with H_2O and extracted with AcOEt. The extract was washed with H_2O, sat. NaClaq and dried over Na_2SO_4. On removal of the solvent, there remained crystals (90 mg), identified with V by comparison of the IR spectra.

Noranisatin ketoaldehyde (VI)

To a soln of II (312 mg) in 70% AcOHaq (6 ml) was added dropwise a soln of $Pb(OAc)_4$ in AcOH (17 ml, concentration, 0·066 mole/l). The mixture was kept at room temp for 4 hr and ethylene glycol (ca. 20 mg) was added. After evaporation of solvent under red. press. H_2O was added to the residue and the mixture was extracted with AcOEt. The extract was washed with H_2O, sat. NaClaq and dried over Na_2SO_4. On removal of the solvent, colorless oily product (ca. 300 mg) was obtained, which on recrystallization from THF afforded plates of VI, 90 mg, m.p. 191–192°: IR bands at 1847, 1767, 1725, 1712 cm^{-1} (THF). (Found: C, 56·88; H, 5·36. $C_{14}H_{16}O_7$ requires: C, 56·75; H, 5·44%.)

Noranisatin ketoacid (VII)

(a) *From* VI: A soln of $KMnO_4$ (140 mg) in 10N H_2SO_4 (20 ml) was gradually added to a soln of VI (200 mg) in AcOH (5 ml)–10N H_2SO_4 (5 ml) at room temp. The mixture was extracted with ether and the extract was washed with H_2O, sat. NaClaq and dried over Na_2SO_4. The colorless oily product (170 mg) obtained on evaporation of the solvent under red. press. was recrystallized from AcOEt–$CHCl_3$ to give needles of VII, m.p. 152–154°; pKa' 4·29 (H_2O); IR bands at 1828, 1751, 1730, 1715 (shoulder) cm^{-1} (KBr); NMR signals (CD_3COCD_3) at 0·94 (3H, d, J = 7), 1·51 (3H, s), 4·66 (2H, q, AB-type, J_{AB} = 6), 4·76 (1H, d, J = 6). (Found: C, 53·78; H, 5·04. $C_{14}H_{16}O_8$ requires: C, 53·84; H, 5·16%.)

(b) *From* III: An aqueous soln of HIO_4 (200 mg/3 ml) was added to a soln of III (100 mg) in MeOH (4 ml). The mixture was stirred at room temp for 10 hr and concentrated under red. press. to afford crystals (40 mg). Recrystallization from $CHCl_3$–AcOEt gave needles, m.p. 152–154°. The identity of the two acids prepared from VI and III was proved by the IR spectra and mixed m.p.

Bromonoranisatinone (VIII)

A soln of Br_2 (160 mg) in AcOH (1 ml) was added to a soln of III (188 mg) in AcOH (10 ml) containing 2 drops of 48% HBraq. The mixture was heated at 70° for 1·5 hr and concentrated under red. press. The residue was chromatographed on silicic acid using $CHCl_3$ as the eluting solvent. Recrystallization from acetone–ether gave crystals of VIII, 150 mg, m.p. 207–211°; IR bands at 1827, 1774, 1767 (shoulder) cm^{-1}

(KBr); the NMR spectrum (Fig. 2); mass 376 and 374 (M^+). (Found: C, 44·76; H, 4·07. $C_{14}H_{15}O_7Br$ requires: C, 44·81; H, 4·03%.)

Reduction of bromonoranisatinone (VIII) *to noranisatinone* (III)

A mixture of VIII (90 mg) and powdered Zn (100 mg) in AcOH (5 ml) was stirred overnight at room temp and refluxed for 1 hr. The mixture was filtered and the filtrate was concentrated under red. press. The residue was dissolved in AcOEt and the soln was washed with H_2O, sat. NaCl aq and dried over Na_2SO_4. On removal of the solvent, there remained crystalline material, which was recrystallized from $CHCl_3$ to give crystals, m.p. 213–215°, identified as III by comparison of the IR spectra and mixed m.p.

Rate of $Pb(OAc)_4$ *consumption by* II, III *and* IV

Samples (3–6 mg) were dissolved in the standardized soln of $Pb(OAc)_4$. Aliquots were analyzed (iodometry) periodically to determine the progress of the oxidation. Results are summarized below.

II: 0·84 mole (2 hr), 0·90 mole (9 hr), 1·02 moles (30 hr).
III: 0·08 mole (2 hr), 0·10 mole (6 hr), 0·11 mole (50 hr).
IV: 0·00 mole (3 hr), 0·00 mole (20 hr).

Noranisatinic acid acetate (IX)

To a methanolic soln of IV (1·675 g/50 ml) was added an aqueous soln of $KHCO_3$ (1·70 g/30 ml). The mixture was kept at 60–70° for 4 hr and then neutralized with 5N H_2SO_4 (3 ml) with cooling. Precipitated K_2SO_4 was once filtered and 5N H_2SO_4 was further added to the filtrate for the complete precipitation of K_2SO_4. Additional K_2SO_4 was filtered and the volume of the acidic filtrate was reduced below 20° under red. press. to ca. 20 ml ($\frac{1}{5}$ of the volume of the filtrate). Crystals (IX) appeared, which were separated by filtration and further concentration of the filtrate afforded a yellow oil. The oily residue was dissolved in AcOEt and the soln was washed with H_2O, sat. NaCl aq and dried over Na_2SO_4. On removal of the solvent, there remained an oily material (420 mg), which was chromatographed on silicic acid (25 g). Fractions (70 ml) eluted by $CHCl_3$ gave a starting compound (IV), 142 mg: fractions (80 ml) eluted with $CHCl_3$–AcOEt (1:1) afforded an oily product (80 mg): elution with AcOEt (40 ml) gave crystals (IX), 32 mg, which were combined with those IX obtained during concentration of the acidic filtrate and recrystallized from AcOEt to afford pure crystals, IX, 103 mg, m.p. 192–194°; p*Ka'* 3·8 (H_2O); IR bands at 1765, 1735 cm^{-1} (KBr). (Found: C, 53·65; H, 6·40. $C_{16}H_{22}O_9$ requires: C, 53·63; H, 6·19%.)

Methyl noranisatinate acetate (X)

Crystals (230 mg) of IX were added to an ethereal solution of CH_2N_2. Crystals (IX) were completely dissolved in the ether soln with evolution of N_2 gas and the soln was allowed to stand overnight. Crystals of X appeared on the walls of the flask and a yellow oil was deposited. Recrystallization of the collected crystals from ether yielded plates of X, 68 mg, m.p. 190–191°; IR band at 1730 (strong) cm^{-1} ($CHCl_3$); NMR signals ($CDCl_3$) at 1·18 (3H, d, J = 6·5), 1·36 (3H, s), 2·06 (3H, s), 3·70 (3H, s), 3·72 (1H, t, J = 2·5), 4·60 (2H, q, AB-type, J = 13·5). (Found: C, 54·91; H, 6·32. $C_{17}H_{24}O_9$ requires: C, 54·83; H, 6·50%.)

Rate of $Pb(OAc)_4$ *consumption by* X

The sample (41·6 mg) was dissolved in the standardized solution (0·046N, 10·0 ml) of $Pb(OAc)_4$. Aliquots were analyzed (iodometry) periodically to determine the progress of the oxidation. Results are summarized below.

0·72 mole (20 hr), 0·91 mole (30 hr), 0·93 mole (42 hr).

4,7,8-*Trimethyldihydrocoumarin* (XI)

(a) *From the ketoacid* (VII). A mixture of VII (215 mg) and red P (164 mg) in freshly distilled HI (8 ml) was refluxed for 23 hr. On cooling, an oily layer separated from the aqueous soln. The mixture was diluted with H_2O (60 ml) and extracted with three 35 ml portions of ether. The deep-red ethereal extracts were washed with H_2O (twice), $Na_2S_2O_3$ aq (three times) and sat. NaCl aq and dried over Na_2SO_4. On removal of the solvent, there remained a yellow liquid (128 mg), which showed a main spot at R_f 0·5 on TLC. The liquid product was purified by preparative TLC (3 plates of the size 20 cm × 20 cm), using $CHCl_3$–CCl_4 (1:2) as the developing agent. Silica gel GF containing XI was extracted with three 20 ml portions of $CHCl_3$ and the combined extracts were concentrated under red. press. to give a slightly

yellow liquid (70 mg), which was chromatographed on silicic acid (3 g) using $CHCl_3$ as the eluting solvent. Colorless liquid (60 mg) was obtained. The above procedure was repeated and the combined colorless liquid (110 mg) was distilled under red. press. to afford pure XI, 80 mg, b.p. 110–112° (2 mm); IR bands at 1775, 1620, 1580 cm^{-1} (liquid); UV absorptions at 265 mμ (ε 550), 275 mμ (shoulder, ε 400) in a neutral solution and at 281 mμ (ε 1600), 294 mμ (ε 1700) in an alkaline solution; NMR signals (see the text); mass 190 (M^+), 175, 162, 148 (base peak); VPC showed a single peak. (Found: C, 75·63; H, 7·55. $C_{12}H_{14}O_2$ requires: C, 75·76; H, 7·42%.)

(b) *Synthesis.* To a mixture of $MeCOCH_2COOEt$ (6 g) and 2,3-dimethylxylenol (6 g) was added gradually conc H_2SO_4 (12 ml) with cooling. The mixture was kept at room temp for 2 days and poured onto ice. A yellow ppt resulted which was filtered, dried and recrystallized from EtOH to afford needles of 4,7,8-trimethylcoumarin. 7 g, m.p. 143–144° (lit.[7] 145°). The trimethylcoumarin (2 g) was catalytically reduced with PtO_2 (450 mg) in AcOH (60 ml); within 2 hr, 1 mole of H_2 was absorbed. After removal of the catalyst, the soln was concentrated under red. press. to give a colorless liquid, which was dissolved in ether (60 ml). The ethereal soln was washed with sat. $NaHCO_3$ aq (3 times), H_2O (twice) and dried over Na_2SO_4. On removal of the solvent, there remained a colorless liquid (2 g), which was distilled under red. press. to afford pure XI, 1·7 g, b.p. 114–115° (2 mm); spectral properties (IR, UV, NMR, mass) and the VPC behavior were in complete agreement with those of XI prepared from the ketoacid VII. (Found: C, 75·50; H, 7·98. $C_{12}H_{14}O_2$ requires: C, 75·76; H, 7·42%.)

4,7-Dimethyl-8-chloromethyldihydrocoumarin (XII)

A soln of VII (1 g) in AcOH (40 ml) saturated with HCl gas was heated in a sealed tube at 130–140° for 3 hr. After cooling, the colored soln was concentrated under red. press. to give a dark-brown oily product, which was left overnight in a vacuum dessicator (KOH pellets as drying reagent). The oily product (970 mg) was chromatographed on silicic acid (23 g), using $CHCl_3$ as the eluting solvent. Crude crystals (230 mg) were recrystallized from EtOH to give needles of XII, 100 mg, m.p. 84–85°; IR bands at 1770, 1615, 1585 cm^{-1} ($CHCl_3$), mass 226 (M^+, Cl = 37), 224 (M^+, Cl = 35), 189 (base peak, $M^+ - Cl$). (Found: C, 64·35; H, 6·02. $C_{12}H_{13}O_2Cl$ requires: C, 64·58; H, 5·83%.)

Conversion of XII *to* XI

In AcOH (5 ml), XII (26 mg) was catalytically reduced with PtO_2 (6 mg); 1 mole of H_2 was absorbed within 1 hr. After removal of the catalyst, the soln was concentrated under red. press. to give a colorless liquid, which was dissolved in benzene (10 ml). The benzene soln was washed with sat. $NaHCO_3$ aq twice and H_2O and dried over Na_2SO_4. On evaporation of the solvent, there remained a colorless liquid, which was purified by preparative VPC. The liquid obtained, proved to be XI by spectral comparison (IR and mass).

Phenol lactone carboxylic acids (XIII, XIV)

An EtOH soln (4 ml) of XII (85 mg) was mixed an aqueous soln (1·5 ml) of NaCN (21 mg). The mixture was kept under reflux for 3 hr, diluted, after cooling, with H_2O (15 ml) and extracted with two 20 ml portions of ether. The aqueous layer was made acidic with dil H_2SO_4 and extracted with ether. The combined ethereal extracts were washed with H_2O, sat. NaCl aq and dried over Na_2SO_4. The solvent was removed and the resulting yellow oil (97 mg) was dissolved in 1N NaOH (4·5 ml) and refluxed for 2 hr. After cooling, the soln was acidified with 2N HCl and extracted with two 20 ml portions of ether. The ether extracts were washed with H_2O and sat. NaCl aq and concentrated under red. press. to give a yellow oil (81 mg), which was dissolved in 2N HCl (3 ml)–THF (7 ml) and refluxed for 2 hr. Complete evaporation of the solvent yielded a yellow powdered material, which was chromatographed on silicic acid (5 g) using $CHCl_3$ as the eluting solvent to afford crude crystals. Recrystallization from benzene–CCl_4 was repeated twice; plates, 25 mg, m.p. 175–180°; IR bands at 1805, 1775, 1710 cm^{-1} (KBr). The crystals proved to be a mixture of XIII and XIV by two spots on TLC (R_f, 0·45, 0·47; $CHCl_3$–MeOH (85:15) as developing agent) and by the IR spectrum. (Found: C, 67·08; H, 6·20. $C_{13}H_{14}O_4$ requires: C, 66·65; H, 6·02%.)

Ketolactone (XV)

(a) To a soln of X (70 mg) in AcOH (20 ml) was added a soln of CrO_3 (70 mg) in AcOH (5 ml). The mixture, after addition of 1 drop of H_2O was heated at 60–70° for 3·5 hr and allowed to stand overnight at room temp. The crystals which appeared were separated by filtration and recrystallized from MeOH to give needles, 43 mg, 226–240° (sublimation). No further crystals were obtained from the filtrate. The

crystals were almost insoluble in $CHCl_3$ and pyridine, and sparingly soluble to MeOH (30 mg/50 ml). IR bands at 1785, 1748, 1733 (broad), 1703 cm^{-1} (Nujol); mass 368 (M^+), 326, 295, 283, 266, 207 (base peak). (Found: C, 54·98; H, 5·52. $C_{17}H_{20}O_9$ requires: C, 55·43; H, 5·47%.)

(b) To a yellow slurry of the CrO_3–pyridine (50 mg–1·7 ml) complex was added dropwise a soln of X (40 mg) in pyridine (1 ml) with stirring. The clear deep-red soln was left overnight and concentrated under red. press. to give a dark-brown residue, to which H_2O was added. The mixture was extracted with AcOEt repeatedly, and the extracts after drying over Na_2SO_4 were concentrated to afford crystals of XV.

Anisatin monoacetate (XVI)

A soln of I (3 g) in Ac_2O (1·2 ml)–pyridine (35 ml) was kept overnight at room temp and concentrated under red. press. to give a colorless oily material. The product was dissolved in ether and the soln was washed with dil H_2SO_4 and sat. NaCl aq and dried over Na_2SO_4. On removal of the solvent there remained crystals, which on recrystallization from AcOEt afforded plates, XVI, 2·84 g, m.p. 173–174°; IR bands at 1825, 1740 (strong) cm^{-1} (KBr); NMR signals ($CDCl_3$) at 1·10 (3H, d, J = 7·0), 1·67 (3H, s), 2·17 (3H, s), 3·86 (1H, s, OH), 4·38 (2H, q, AB-type, J_{AB} = 7·0), 4·30 (1H, s), 4·50 (1H, m,), 6·01 (1H, q, J_{AX} = 6·0, J_{BX} = 8·5). (Found: C, 55·09; H, 5·85. $C_{17}H_{22}O_9$ requires: C, 55·13; H, 5·99%.)

Oxidation of anisatin monoacetate (XVI)

A soln of XVI (2·44 g) in AcOH (30 ml) was mixed with an aqueous AcOH soln (AcOH–H_2O, 30 ml–12 ml) of $KMnO_4$ (3 g). The mixture was warmed at 35–40° for 3 hr. A dark-brown ppt appeared. The mixture was concentrated, diluted with H_2O and extracted with AcOEt. The extracts were washed with H_2O, sat. NaCl aq and dried over Na_2SO_4. On removal of the solvent under red. press. there remained a solid, which on recrystallization from ether afforded plates, 1·48 g, m.p. 186–188°. The product proved to be IV by the IR spectra.

Anisatin triacetate (XVII)

A soln of I (700 mg) in Ac_2O (20 ml)–pyridine (2·5 ml) was kept at room temp for 6 days, heated on a water-bath (ca. 90°) for 1 hr, and poured into H_2O (200 ml) to give a white solid, which was filtered and recrystallized from $CHCl_3$–ether; needles, 640 mg, m.p. ca. 230° (sublimation); IR bands at 1842, 1767, 1744 (strong) cm^{-1} (KBr); NMR spectrum (Fig. 3); mass 454 (M^+), 412, 394. (Found: C, 55·29; H, 5·99. $C_{21}H_{26}O_{11}$ requires: C, 55·50; H, 5·76%.)

Anisatin monobenzoate (XVIII)

A soln of I (150 mg) in benzoyl chloride (170 mg)–pyridine (2 ml) was kept at room temp for 8 days, warmed at 60–70° for 40 min, poured into H_2O (ca. 50 ml) and left overnight. A precipitated solid was separated by decantation, dissolved in AcOEt and the soln was washed with sat. $NaHCO_3$ aq, H_2O (twice) and dried over Na_2SO_4. On removal of the solvent, there remained a yellow oily residue, which gradually crystallized. Recrystallization from $CHCl_3$–ether afforded needles, XVIII, m.p. 229–231°; IR bands at 1831, 1745, 1718 cm^{-1} (KBr). (Found: C, 60·96; H, 5·70. $C_{22}H_{24}O_9$ requires: C, 61·10; H, 5·59%.)

Anisatin monotosylate (XIX)

A pyridine soln (16 ml) containing I (1·2 g) and tosyl chloride (1·6 g) was kept at room temp for 2 days and concentrated under red. press. to give a yellow oily material, to which a large amount of H_2O was added. After standing 3–4 hr, the oily material gradually solidified. The solid was filtered, washed with H_2O, dried and recrystallized from $CHCl_3$–benzene to afford needles, 1·5 g, m.p. 194–195°; IR bands at 1825, 1745 cm^{-1} (KBr). (Found: C, 54·29; H, 5·38. $C_{22}H_{26}O_{10}S$ requires: C, 54·76; H, 5·43%.) No consumption of $Pb(OAc)_4$ was observed under conditions employed in I.

Anisatin carbonate (XX)

Phosgene gas was passed through the soln of I (3 g) in THF (65 ml)–pyridine (6 ml) cooled in an ice-bath, for 1·5 hr. During the reaction, a yellow oil was deposited. The mixture was concentrated under red. press. to give a syrup. Water (40 ml) was added and the mixture was extracted with AcOEt 3 times. The extracts were washed with H_2O sat. $NaHCO_3$ aq and sat. NaCl aq and dried over Na_2SO_4. On evaporation of the solvent under red. press. an oily residue was obtained, which gradually crystallized. A small amount of $CHCl_3$ was added and crude crystals were filtered and recrystallized to give needles, 1·48 g, m.p. 218–220°; IR bands at 1820 (broad), 1750 (weak), 1725 cm^{-1} (KBr); NMR signals (CD_3COCD_3) at 1·05 (3H, d, J = 6·5), 1·49 (3H, s), 4·14 (1H, s, OH), 4·29 (1H, t, J = 2·5), 4·36 (2H, q, AB-type, J_{AB} = 7), 4·38

(1H, d, $J = 4$), 5·32 (1H, q, $J_{AX} = 2$, $J_{BX} = 7·5$), 5·55 (1H, d, $J = 4$, OH). (Found: C, 53·83; H, 5·24. $C_{16}H_{18}O_9$ requires: C, 54·24; H, 5·12%.)

Oxidation of anisatin carbonate (XX)

(a) To a soln of XX (408 mg) in AcOH (14 ml) was added a soln of $KMnO_4$ (800 mg) in AcOH (8 ml)–H_2O (2 ml). The mixture was kept at 30–40° for 3 hr and left for 10 hr. A dark-brown ppt appeared. The mixture was concentrated and after H_2O was added, extracted with AcOEt repeatedly. The combined extracts were washed with H_2O, sat. NaCl aq and dried over Na_2SO_4. On removal of the solvent, a colorless oily residue was obtained, which gradually crystallized. Recrystallization from AcOEt gave needles of V, 212 mg, m.p. 222–223°.

(b) A soln of I (200 mg) in AcOH (6 ml) was mixed with a soln of CrO_3 (70 mg) in AcOH (2 ml)–H_2O (0·5 ml). The mixture was kept at room temp overnight, heated at 50–60° for 5 hr, concentrated under red. press. and diluted with H_2O (10 ml). The green aqueous soln was extracted with three 30 ml portions of AcOEt and the combined colorless extracts were dried over Na_2SO_4. The solvent was removed under red. press. to give crystals, which were chromatographed on silicic acid using $CHCl_3$–AcOEt (8:1) as the eluting solvent. Recrystallization from $CHCl_3$–AcOEt gave needles, 110 mg, m.p. 236–238° (sublimation), which were identified as V by the IR spectral comparison.

Anhydroanisatin (XXI)

A soln of XIX (1·37 g) in pyridine (34 ml) was refluxed for 14 hr, concentrated, after cooling, under red. press., diluted with H_2O (20 ml) and extracted with three 20 ml portions of AcOEt. The combined extracts were washed with sat. $NaHCO_3$ aq (twice), $CdCl_2$ aq (twice), H_2O (twice) and sat. NaCl aq and dried over Na_2SO_4. On removal of the solvent under red. press., an oily product was obtained, which gradually crystallized. Recrystallization from AcOEt–benzene afforded needles, 700 mg, m.p. 180–181° (sealed tube); IR bands at 1823, 1760 (shoulder), 1749 cm^{-1} (KBr); NMR spectrum (Fig. 4); mass 310 (M^+). (Found: C, 57·82; H, 5·81. $C_{15}H_{18}O_7$ requires: C, 58·06; H, 5·85%.)

Preparation of XXI *in the presence of deuterium oxide*

A soln of XIX (59 mg) in pyridine (7 ml)–D_2O (0·3 ml) was kept at reflux for 20 hr and concentrated under red. press. An AcOEt soln of the residue was washed with 1N HCl, $NaHCO_3$ aq, H_2O and sat. NaCl aq and dried over Na_2SO_4. On evaporation of the solvent an oil (35 mg) was obtained, which was chromatographed on silicic acid (1 g) using $CHCl_3$ as the eluting solvent to afford crystals (30 mg). Recrystallization from AcOEt–ether yielded XXI; the intensity ratio, $M + 1/M = 0·5$ in the mass spectrum.

Noranisatin ketoaldehyde methanolate (XXII)

To a soln of II (500 mg) in MeOH (10 ml) was added an aqueous soln of HIO_4 ($HIO_4 \cdot 2H_2O$, 1500 mg/6 ml). The mixture was kept at room temp for several hr, and concentrated under red. press. Crude crystals (ca. 190 mg) were deposited and recrystallized from $CHCl_3$ to afford XXII, m.p. 178–180°; IR bands at 1825, 1786 cm^{-1} ($CHCl_3$); NMR signals (CD_3COCD_3) at 1·00 (3H, d, $J = 6·5$), 1·55 (3H, s), 2·91 (1H, s, OH), 3·38 (3H, s), 4·31 (2H, q, AB-type, $J_{AB} = 6$), 4·41 (1H, q, $J_{AX} = 1·5$, $J_{BX} = 5$), 5·08 (1H, m), 5·70 (1H, broad, OH). (Found: C, 54·55; H, 6·10. $C_{15}H_{20}O_8$ requires: C, 54·87; H, 6·14%.)

Anisatin sulfite (XXIII)

Dried pyridine (0·6 ml) was added dropwise to a suspension of I (1 g) in freshly distilled $SOCl_2$ (9 g), and I was gradually dissolved. The soln was warmed at 45–50° for 1 hr and concentrated under red. press. A $CdCl_2$ soln was added to the residue and the mixture was extracted with three 20 ml portions of AcOEt. The combined extracts were washed with H_2O and sat. NaCl aq and dried over Na_2SO_4. On evaporation of the solvent under red. press. a yellow solid was obtained, which was chromatographed over acid-washed alumina. Fractions eluted with $CHCl_3$–AcOEt (3:1) afforded a crystalline product, which upon recrystallization from $CHCl_3$–AcOEt afforded needles, XXIII, 160 mg, m.p. 238–239°; IR bands at 3540, 1825, 1745 cm^{-1} (KBr). (Found: C, 48·08; H, 4·98. $C_{15}H_{18}O_9S$ requires: C, 48·12; H, 4·84%.)

Anhydroanisatin sulfite (XXIV)

Dried pyridine (0·5 ml) was added to I (1 g) suspended in freshly distilled $SOCl_2$ (10 g), and I was dissolved. The soln was refluxed for 3 hr and concentrated under red. press. to afford a red-brown oily

product. An aqueous $CdCl_2$ soln was added and the mixture was extracted with three 20 ml portions of AcOEt. The combined extracts were washed with H_2O and dried over Na_2SO_4. On removal of the solvent under red. press., an oily residue was obtained, which was treated with ether to give crystals after 1 day. Crude crystals (410 mg) were chromatographed over acid-washed alumina; early fractions eluted by $CHCl_3$–AcOEt (4:1) afforded XXIV (250 mg) and later fractions gave XXIII (90 mg). Recrystallization from $CHCl_3$–ether afforded needles of XXIV, m.p. 204–206°; IR bands at 1835, 1760, 1740 (shoulder) cm^{-1} (KBr). (Found: C, 50·33; H, 4·07. $C_{15}H_{16}O_8S$ requires: C, 50·55; H, 4·52%.)

Neoanisatin diacetate (XXVI)

A soln of XXV (150 mg) in Ac_2O (1 ml)–pyridine (0·3 ml) was kept at 40° for 20 days and concentrated under red. press. Water was added to the residue, which gradually crystallized (170 mg). Recrystallization from $CHCl_3$–AcOEt yielded needles, m.p. 194–195°; IR bands at 1835, 1765, 1745 (shoulder) cm^{-1} (KBr); NMR spectrum (Fig. 5). (Found: C, 57·60; H, 6·40. $C_{19}H_{24}O_9$ requires: C, 57·57; H, 6·10%.)

Norneoanisatin (XXVII)

(a) A soln of XXV (10·7 g) in AcOH (410 ml) was mixed with an aqueous $KMnO_4$ soln (11·1 g/140 ml). After standing overnight at room temp, the mixture was heated at 60–70° for 1·5 hr, and excess $KMnO_4$ (and MnO_2) was reduced with $NaHSO_3$ aq. The yellow clear soln was concentrated under red. press. to give a syrup, to which H_2O was added. The mixture was extracted with four 80 ml portions of AcOEt. The combined extracts were washed with sat. $NaHCO_3$ aq (4 times) and sat. NaCl aq and dried over Na_2SO_4. On removal of the solvent under red. press., a yellow residue was obtained, which partly crystallized (8·5 g). Recrystallization from $CHCl_3$ gave plates, XXVII, 3·6 g, 153–155°. The mother liquor was concentrated under red. press. to give an oil (ca. 5 g), which was chromatographed on silicic acid (80 g) using $CHCl_3$ as the eluting solvent; crystals of XXVII, 2·5 g, m.p. 154–155°; total amount, 6·1 g. Further recrystallization from $CHCl_3$ afforded pure XXVII, m.p. 155–156°; IR bands at 1832, 1776 cm^{-1} ($CHCl_3$); NMR spectrum (Fig. 6). (Found: C, 59·20; H, 6·43. $C_{14}H_{18}O_6$ requires: C, 59·56; H, 6·43%.)

(b) A soln of XXV (625 mg) in AcOH (20 ml) was mixed with a soln of CrO_3 (200 mg) in AcOH (6 ml)–H_2O (2 ml). The mixture was left overnight, warmed at 50–55° for 5 hr and concentrated under red. press. to give a green solid. Water (10 ml) was added and the mixture was extracted with two 50 ml portions of AcOEt. The extracts were washed with sat. NaCl aq, dried over Na_2SO_4 and concentrated under red. press. to afford an oily product, which was chromatographed on silicic acid using $CHCl_3$ as the eluting solvent; early fractions contained XXVII. Recrystallization of the combined crystals gave plates, ca. 200 mg, m.p. 155–156°.

Lactone diacid (XXVIII)

(a) To a suspension of XXV (3 g) in 6N H_2SO_4 (150 ml) was added gradually an aqueous CrO_3 soln (6 g/50 ml) and the mixture was kept at 90–95° for 4 hr. During this period, crystals of XXV were dissolved. To the mixture was added further a soln of CrO_3 (6 g/40 ml) and the mixture was refluxed for 5 hr. After standing overnight, the volume of the mixture was reduced by concentration under red. press. to ca. 100 ml. The concentrated mixture was extracted with six 50 ml portions of AcOEt and the combined extracts were washed with H_2O (100 ml × 2) and with three 50 ml portions of sat. $NaHCO_3$ aq. The AcOEt layer was dried over Na_2SO_4 and concentrated under red. press. to give an oily material (ca. 200 mg), which was chromatographed on silicic acid (10 g) using $CHCl_3$ as the eluting solvent: crystals (120 mg) were obtained, which proved to be XXVII by IR spectral comparison. The $NaHCO_3$ aq layers were combined, made acidic with dil HCl and extracted with four 50 ml portions of AcOEt. The extracts were washed with H_2O, sat. NaCl aq and dried over Na_2SO_4. On removal of the solvent a yellow viscous oil (445 mg) was obtained, which was chromatographed over silicic acid (20 g); elution with $CHCl_3$–AcOEt (4:1) gave a crystalline residue (284 mg), which on recrystallization from $CHCl_3$–acetone afforded needles, XXVIII, 150 mg, m.p. 202–204°; pKa′ 3·6 and 4·8 (H_2O); IR bands at 1803, 1700 cm^{-1} (KBr); NMR signals (CD_3COCD_3) at 1·08 (3H, d, J = 6·5), ~2·2 (5H, m, measured in pyridine), 2·86 (2H, q, AB-type, J = 18), 9·3 (2H, s, OH). (Found: C, 52·28; H, 5·37; O, 41·74. $C_{10}H_{12}O_6$ requires: C, 52·63; H, 5·30; O, 42·07%.)

(b) To a suspension of XXVII (2 g) in 6N H_2SO_4 (90 ml) was added an aqueous CrO_3 soln (4 g/30 ml) and the mixture was heated at 90–100° for 20 hr. The mixture was concentrated and extracted with AcOEt. The same procedure described in (a) was applied in here and pure crystals of XXVIII were obtained, 160 mg.

(c) A soln of lactone diacid (XXVIII; 50 mg) in Ac_2O (3 ml)–pyridine (0·04 ml) was gently refluxed for

5 hr and concentrated under red. press. to give a colorless oil, which crystallized gradually; IR bands at 1865, 1800 (strong) cm^{-1} (KBr). The crystalline product is liable to be hydrolyzed to regenerate XXVIII.

Norneoanisatinic acid (XXIX)

To a suspension of XXVII (2·4 g) in H_2O (20 ml) was added 5% $Ba(OH)_2$ aq (60 ml). The mixture was stirred at 25–28° for 7 hr to become a yellow clear soln. The soln was made acidic with H_2SO_4 and the resulting ppt was extracted with AcOEt. The extract was washed with H_2O, dried over Na_2SO_4 and concentrated under red. press. to give a residue (2·5 g), which was chromatographed on silicic acid (40 g), using $CHCl_3$ as the eluting solvent; fractions eluted by $CHCl_3$–AcOEt (5:1) gave unreacted material, XXVII, 424 mg; fractions eluted with $CHCl_3$–AcOEt (1:1) afforded a product, which on recrystallization from MeOH–AcOEt gave needles, XXIX, 62 mg, m.p. 212–213°, p*Ka'* 3·8 (H_2O); IR bands at 1723, 1712 cm^{-1} (KBr). (Found: C, 55·90; H, 6·72. $C_{14}H_{20}O_7$ requires: C, 55·99; H, 6·71%.)

Methyl norneoanisatinate (XXX)

Crystals of XXIX (80 mg) were treated with an excess of ethereal CH_2N_2. The soln was left overnight, when crystals appeared in the flask. The crystals were collected and recrystallized from ether, 34 mg, m.p. 205–208°; IR bands at 1738 cm^{-1} ($CHCl_3$); NMR spectrum (Fig. 7). (Found: C, 57·11; H, 7·03. $C_{15}H_{22}O_7$ requires: C, 57·31; H, 7·06%.) Since XXX reacted with $Pb(OAc)_4$, the rate of consumption of $Pb(OAc)_4$ was determined: 0·72 mole (20 hr), 0·93 mole (30 hr), 0·95 mole (40 hr).

Neo-ketolactone (XXXI)

To a soln of XXX (40 mg) in AcOH (14 ml) was added a soln of CrO_3 (50 mg) in AcOH (1 ml). The mixture, after addition of 1 drop of H_2O was heated at 60–70° for 3·5 hr and left overnight at room temp. Crystals precipitated in the soln were collected and recrystallized from EtOH to give needles, 17 mg, m.p. 228–235° (sublimation). The crystals were sparingly soluble to acetone, pyridine, THF and AcOEt; IR bands at 1775, 1735, 1725, 1701 cm^{-1} (KBr). (Found: C, 58·01; H, 5·83. $C_{15}H_{18}O_7$ requires: C, 58·06; H, 5·85%.)

Hexahydro norneoanisatin (XXXII)

To a soln of XXVII (1·114 g) in THF (50 ml, distilled from LAH) was added LAH (0·5 g) in small portions. The mixture was refluxed for 4 hr. Excess LAH and complexes were decomposed under cooling by dropwise addition of wet THF. The ppt was removed by filtration and the filtrate was concentrated at room temp under red. press. to afford an oily product. AcOEt was added to the residue and insoluble materials were filtered and the filtrate was left at room temp for 10 days to give crystals. Recrystallization from acetone afforded needles, 0·371 g, m.p. 230–235° (sublimation); IR band at 3400 (strong) cm^{-1}, no C=O (KBr); NMR spectrum (Fig. 8); mass 270 ($M^+ - 18$). (Found: C, 58·29; H, 8·35. $C_{14}H_{24}O_6$ requires: C, 58·31; H, 8·39%.)

Hexahydronorneoanisatin diacetate (XXXIII)

A soln of XXXII (80 mg) in Ac_2O (0·05 ml)–pyridine (2 ml) was kept at room temp for 12 hr and diluted with H_2O (10 ml). After standing for 3 hr, the mixture was concentrated under red. press. to give a crystalline product, which was recrystallized from pet. ether; needles, 64 mg, m.p. 156–157°; IR band at 1745 cm^{-1} ($CHCl_3$); NMR spectrum (Fig. 8). (Found: C, 57·91; H, 7·57. $C_{18}H_{28}O_8$ requires: C, 58·05; H, 7·58%.)

Neo-ketolactone monoacetate (XXXIV)

Lead tetraacetate (100 mg) was added to a soln of XXXIII (63 mg) in AcOH (20 ml) and the mixture was left at room temp for 48 hr. Water (25 ml) and sat. $Na_2S_2O_3$ aq (4 ml) were added and the mixture was extracted with AcOEt repeatedly. The extract was washed with H_2O, dried over Na_2SO_4 and concentrated to give an oily residue (57 mg). This $Pb(OAc)_4$ cleavage product was dissolved in AcOH (20 ml) and the soln was mixed with a soln of CrO_3 (70 mg) in AcOH (8 ml) containing several drops of H_2O. The mixture was warmed at 50–60° for 3 hr, concentrated, diluted with H_2O (20 ml) and extracted with AcOEt (20 ml). The extract was washed with H_2O and dried over Na_2SO_4. On evaporation of the solvent, an oily product was obtained, which on addition of pet. ether crystallized. Recrystallization from AcOEt afforded needles, 32 mg, m.p. 185–186°; IR bands at 1778, 1745, 1725, 1701 cm^{-1} and no OH bands ($CHCl_3$); NMR spectrum (Fig. 9); mass 324 (M^+). (Found: C, 59·28; H, 6·25. $C_{16}H_{20}O_7$ requires: C, 59·25; H, 6·22%.)

Acknowledgements—The authors wish to express their thanks to the National Institutes of Health (Grant GM-7969) for generous support in this project, to the Toyo Rayon Science Foundation for purchasing a mass spectrometer and to Parke, Davis and Co., Ann Arbor, Mich., U.S.A. for the fellowships (to K.Y., S.T. and S.N.). We wish to express our gratitude to Takeda Chemical Industries, Ltd., and Nihon Denshi for running the NMR spectra.

REFERENCES

[1] Some of these results were reported as preliminary communications: [a] K. Yamada, S. Takada, S. Nakamura and Y. Hirata, *Tetrahedron Letters* 4785 (1965); [b] N. Sakabe, Y. Hirata, A. Furusaki, Y. Tomiie and I. Nitta, *Ibid.* 4795 (1965); [c] K. Yamada, S. Takada, S. Nakamura and Y. Hirata, *Ibid.* 4797 (1965); [d] S. Takada, S. Nakamura, K. Yamada and Y. Hirata, *Ibid.* 4739 (1966); and were presented at the *4th IUPAC Symposium on the Chemistry of Natural Products*, Stockholm, June (1966). The partial structure of noranisatin was reported: [e] S. Takada, S. Nakamura, K. Yamada and Y. Hirata, *Nippon Kagaku Zasshi* (*J. Chem. Soc. Japan, Pure Chem. Sect.*) **87**, 166 (1966); [f] S. Nakamura, K. Yamada and Y. Hirata, *Ibid.* **87**, 171 (1966).

[2] cf. S. Y. Chen, *Am. J. Pharm.* **101**, 676 (1929), for a review of the early literature.

[3] e.g. J. F. Eykman, *Pharm. J. and Trans.* **11**, 1046 (1881).

[4] J. F. Lane, W. T. Koch, N. S. Leeds and G. Gorin, *J. Am. Chem. Soc.* **74**, 3211 (1952): cf. S. Kawano and A. Matsuo, *Yakugaku Zasshi* **78**, 1220 (1958).

[5] cf. K. Nakanishi, *Infrared Absorption Spectroscopy* p. 42. Holden-Day, San Francisco (1962).

[6] cf. J. H. Bowie, S.-O. Lawesson, G. Schroll and D. H. Williams, *J. Am. Chem. Soc.* **87**, 5742 (1965).

[7] R. N. Lacey, *J. Chem. Soc.* 854 (1954).