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ANISATINIC ACID AND ISOANISATINIC ACID, ISOMERIZATION PRODUCTS OF ANISATIN

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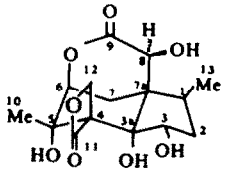
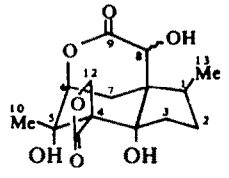
Abstract—Anisatin (I), a toxic compound isolated from *Illicium Anisatum* L. undergoes a base-catalysed isomerization to anisatinic acid (IIIa). Neoanisatin (II) undergoes a similar isomerization to neoanisatinic acid (XVI). Thermal isomerization of I results in formation of isoanisatinic acid (XIX). The mechanistic features of the two types of isomerization have been discussed in detail.

INTRODUCTION

THE structures of anisatin (I) and neoanisatin (II), toxic compounds, obtained from *Illicium Anisatum* L were recently reported.[1] In 1952, Lane *et al.*[2] found that a rapid isomerization of anisatin occurs with alkali to afford anisatinic acid (III). Assuming the presence of an anhydride group in anisatin, Lane concluded that the isomerization is a simple hydrolysis of the anhydride group followed by lactonization of one of the resulting two carboxyl groups.[2] Since it is now unequivocally established that anisatin (I) contains two lactone rings and no anhydride group, Lane's explanation must be revised and in the present paper, re-examination of the isomerization is described. It was found that neoanisatin (II), like anisatin is isomerized to neoanisatinic acid (XVI). The structures of the isomerized acids are interesting from the physiological viewpoint, because both acids are no longer toxic.



I



II

A thermal isomerization of anisatin (I) occurs under controlled conditions to isoanisatinic acid (XIX). The results will be discussed in details on both types of isomerization, a base-catalysed one and a thermally induced one.

Base-catalysed isomerization

1 *Structure of anisatinic acid* (III). In spite of the presence of four OH groups and two lactones, anisatin (I) is stable to a variety of acids.[1e] In contrast, various alkalis cause isomerization of anisatin under mild conditions to give a monobasic acid, anisatinic acid (III), $C_{15}H_{20}O_8$. Anisatin is immediately soluble in aqueous potassium

hydroxide to give the isomerized product III. Furthermore, Lane reported isomerization of anisatin by heating a methanolic solution in a sealed tube.[2] Anisatinic acid (III) is stable to acids and bases.

While anisatin (I) shows two carbonyl bands at 1826 (β-lactone) and 1739 cm^{-1} (δ-lactone) (in Chf), [1c, 1e] anisatinic acid (III) exhibits bands at 1757 and 1725 cm^{-1} (in THF),* and lacks a band due to a β-lactone. There are significant differences in the NMR spectra of anisatin (I) and anisatinic acid (III), as shown in Fig. 1.

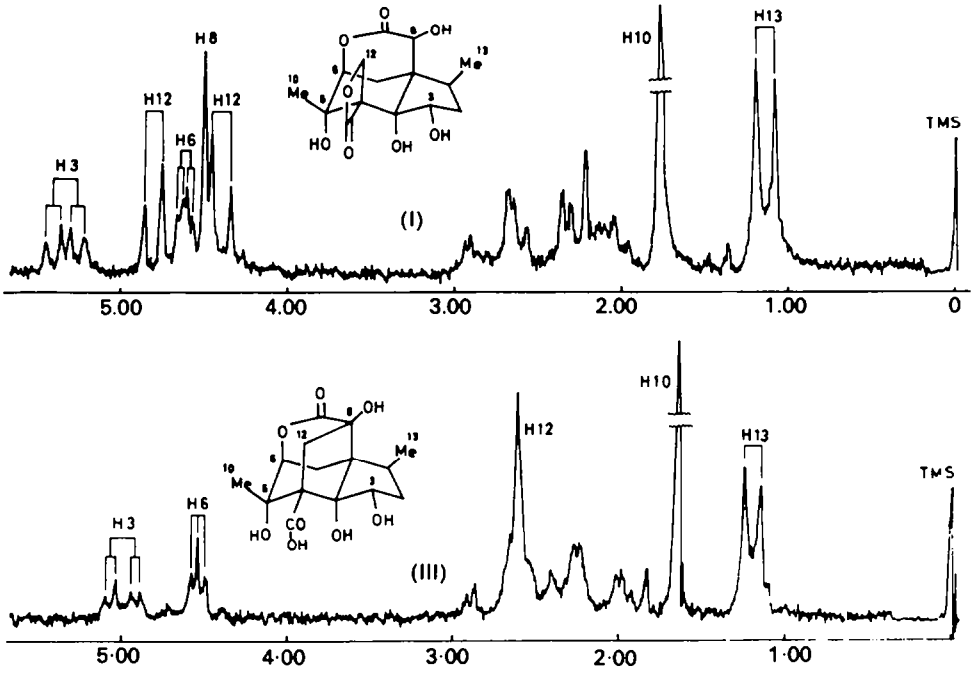


FIG. 1. The NMR spectra of anisatin (I) and anisatinic acid (III): ppm from internal TMS at 60 Mc in trifluoroacetic acid.

In the NMR spectrum of III, signals of the C-8 proton (1H, 4·50 ppm) and the C-12 methylene protons (2H, 4·62 ppm, AB-type, J = 7·0 c/s) present in I are absent and instead a singlet (2H) newly appears at 2·60 ppm. Anisatinic acid (III) consumes one mole of periodic acid rapidly. On treatment with ethereal diazomethane, III afforded methyl anisatinate (IV),[2] which also consumes one mole of periodic acid. The methylester IV gives methyl anisatinate carbonate (V), with phosgene in THF and pyridine. Acetylation of IV with acetic anhydride and pyridine at 95° affords methyl anisatinate diacetate (VI).

The periodic acid consumption by IV and formation of the 5-membered ring carbonate, V indicates the presence of a *cis* 1,2-glycol in anisatinic acid. Since two OH groups are present in methyl anisatinate carbonate (V) as revealed by hydrogen–deuterium exchange, using deuterium oxide in the NMR spectrum (Experimental), anisatinic acid (III) contains four OH groups and one carboxyl group.

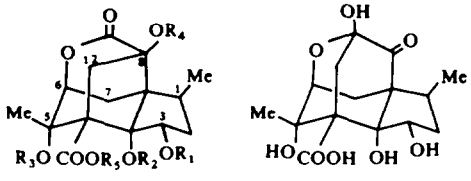
*Owing to poor solubility of III in Chf, the IR spectrum was measured in a THF soln. In general a carbonyl band taken in THF appeared at a higher frequency by 10–20 cm^{-1} than that in Chf. cf. carbonyl bands of IV in Experimental.

Inspection of the NMR spectrum of III reveals that two carbons, C-8 and C-12 of I are involved in the isomerization: a singlet of the C-8 proton in I is absent and a quartet due to the C-12 methylene protons of the type —O—CH_2—C— in I is transformed to a singlet of the type —C—CH_2—C—.

The NMR spectral evidence described above, coupled with the fact that a β-lactone is absent in III suggests the following intramolecular reaction for the isomerization.

The formation of anisatinic acid anhydrodiacetate (VII; *vide infra*) suggests that the carboxyclic 6-membered ring in I remains intact* during isomerization and is present in anisatinic acid (III). This evidence shows that a carboxyl group arising from the β-lactone cannot be lactonized and must be an acidic function of III.

In accordance with these results—(i) one carboxyl and four OH-groups are present in III; (ii) the carboxyl group is derived from the β-lactone of I; (iii) a *cis* 1,2-glycol is present in III; (iv) the carbon skeleton (a 6-membered ring) of I is retained in III; (v) at least C-8 and C-12 are involved in the isomerization—structures IIIa and IIIb are possible for anisatinic acid.



IIIb

IIIa: $R_1 = R_2 = R_3 = R_4 = R_5 = H$
IV: $R_1 = R_2 = R_3 = R_4 = H$; $R_5 = Me$
V: $R_1, R_2 =$ >CO; $R_3 = R_4 = H$; $R_5 = Me$
VI: $R_1 = R_3 = Ac$; $R_2 = R_4 = H$; $R_5 = Me$
VIII: $R_1 = Ac$; $R_2 = R_3 = R_3 = R_5 = H$
IX: $R_1 = CHO$; $R_2 = R_3 = R_4 = R_5 = H$

* There is a possibility that a carbon skeleton of I, an indane system was cleaved by a retro-aldol process, resulting in formation of a keto group at C-3a or C-5, because OH groups on C-5 and C-3a are located beta to the carbonyl function of a β-lactone. However, in order to produce anisatinic acid with four OH groups, an intramolecular aldol condensation must occur between a keto group at C-3a or C-5 and a carbanion (or its equivalent) at C-8 or C-9 (formation of a carbanion at C-9 would be possible through enolization of a δ-lactone and subsequent ketonization at C-8, of the resulting ene-diol intermediate). All the possible structures from these considerations are inconsistent with chemical and spectral properties of III.

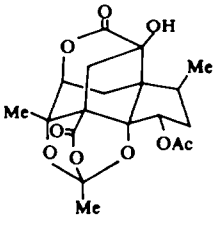
The ORD measurement of anisatinic acid (III) shows a negative plain curve. In the IR spectrum, a sodium salt of III, obtained by heating in an alkaline solution, exhibits a strong broad band at 1590 cm^{-1} and no carbonyl absorptions in the region higher than 1600 cm^{-1}. Anisatinic acid (III) is regenerated from the sodium salt on acidification with dilute hydrochloric acid.

These spectral findings exclude the keto-carboxylic acid structure, IIIb, for anisatinic acid. Thus, anisatinic acid must be the lactone-carboxylic acid, IIIa.

As already mentioned, methyl anisatinate (IV) carrying one secondary OH group affords a diacetate (VI) with acetic anhydride and pyridine. The OH groups on C-3a and C-5 in IV are acetylated to give VI since in anisatin and its derivatives, a tertiary OH group on C-5 is readily acetylated using acetic anhydride and pyridine if two tertiary OH groups on C-3a and C-5 are free.[3] In contrast to the methyl ester IV, anisatinic acid (III) on treatment with acetic anhydride and pyridine gives a neutral compound, anisatinic acid anhydrodiacetate (VII), containing only one OH group, detected by the NMR spectrum.

The anhydrodiacetate (VII) is readily reconverted to the acid, III by hydrolysis employing dilute hydrochloric acid. In the IR spectrum, VII shows bands (1793, 1760 cm^{-1}) due to two carbonyl functions originally present in III and only one acetate band (1735 cm^{-1}). In addition to the signals of a secondary Me (1·13 ppm, d, J = 6 c/s) and a tertiary Me (1·39 ppm, s), two Me signals at 1·81 ppm (s) and at 2·08 ppm (s) are present in the NMR spectrum of VII. While the signal at 2·08 ppm evidently arises from an acetate group, the one at 1·81 ppm located at somewhat higher field than that of a normal acetate must be due to an ortho acetate, the signal of which usually appears at ca. 1·8 ppm.[6]

Since the anhydrodiacetate VII contains one OH group and is neutral, two OH groups and a carboxyl group of III are involved in the formation of the ortho acetate moiety of VII. The structure of anisatinic acid anhydrodiacetate is, therefore, represented by VIIa. In order to form the ortho acetate from two OH groups and



VIIa

a carboxyl group, the carbon skeleton of anisatin (I) must be retained in anisatinic acid (III).

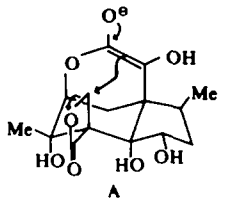
Anisatinic acid (III), when refluxed in acetic acid in the presence of *p*-toluenesulfonic acid affords anisatinic acid acetate (VIII). The NMR spectrum of VIII corresponds with that of anisatinic acid (III) except that the signal of the C-3 proton has moved to lower field by 1·14 ppm on acetylation. Thus, the OH group on C-3 has been acetylated. Similarly, under reflux in formic acid anisatinic acid (III) gives anisatinic acid formate (IX).

Potassium permanganate oxidation of methyl anisatinate (IV), yields methyl

anisatinate ketone (X), the C-3 secondary OH group in IV being oxidized to form a keto group in X.

2 *The formation of anisatinic acid* (III). In anisatin (I), serious steric repulsions must exist between C-8 and C-12 and also between C-12 and a δ-lactone ether oxygen, owing to the 1,3-diaxial relationship. The δ-lactone does not hydrolyse readily since such hydrolysis would produce an additional non-bonding interaction between C-8 and an oxygen on C-6. A β-lactone in general is readily hydrolysed with a base. In anisatin (I), however, the formation of a hydroxymethyl group by hydrolysis of the β-lactone would be unfavourable, because the 1,3-diaxial interactions involving the hydroxymethyl group would be larger in comparison with those due to the methylene group of the β-lactone.* The steric effects discussed above, presumably making hydrolysis of the lactones in I disadvantageous, seem to be responsible for the isomerization of I to III, which would reduce the non-bonding interactions present in I.

By the action of base on I, an enolate A† (or a carbanion) would be formed. In the enolate, non-bonding interaction between C-12 and the OH on C-8 present in I must be reduced, facilitating the formation of the intermediate A. The following



A

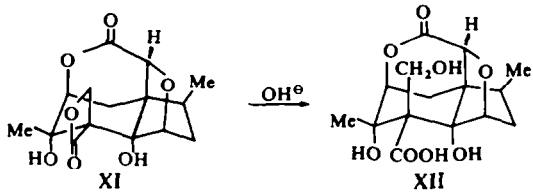
evidence supports the formation of the enolate. On heating anisatin (I) in pyridine containing deuterium oxide (5%), deuteration occurs to some extent (ca. 25%).[1e] While a β-lactone undergoes hydrolysis under basic conditions by acyl-oxygen fission,[5] substitution occurs in general at the β-carbon of a β-lactone with a variety of nucelophiles. Once the enolate A is formed, a reaction of this type would occur intramolecularly. The enolate A, by formation of a new bond between C-8 and C-12 as indicated by arrows in A‡ would yield anisatinic acid.

The following evidence reveals an important role played by the C-8 hydrogen in the isomerization of I to III. In anhydroanisatin (XI)[1e] formation of an enolate such as A would be difficult on steric grounds. On treatment of XI with alkali, no isomerization occurs but hydrolysis of the β-lactone takes place to give anhydroanisatic acid (XII). In noranisatin monoacetate (XIV), a derivative without the C-8 hydrogen,

* Having a free rotation about the C_4–C_{12} bond, the hydroxymethyl group is greater in bulkiness as an axial substituent on C-4 than the methylene group of the β-lactone.

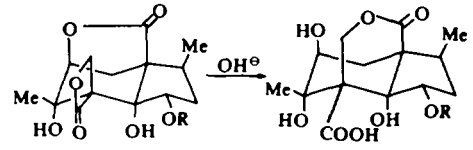
† Owing to the presence of the C-8 OH, formation of a carbanion on C-8, though accelerated by the adjacent δ-lactone carbonyl seems to be unfavourable. Examples indicating the formation of similar intermediates, however, were reported: R. B. Woodward and E. G. Kovach, *J. Am. Chem. Soc.* 72, 1009 (1950) and Refs cited therein.

‡ During the conversion of I to the intermediate A, the β-lactone was assumed to be intact. According to the Lane's result,[2] the isomerization of I by heating in methanol at 120° occurred to afford III together with a small amount of the methylester (IV). Formation of IV would be rationalized by assuming a prior methanolysis of the β-lactone followed by isomerization, which occurred only to a minor extent.



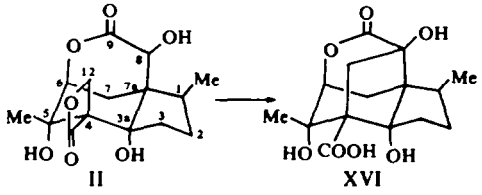
XI XII

hydrolysis occurs to afford noranisatinic acid monoacetate (XV).[1a, 1e] Thus, it is clearly indicated that the C-8 hydrogen in I is readily removed by alkali and plays a role of vital importance in the isomerization.



XIII: R = H
XIV: R = Ac
XV: R = Ac

3 *Neoanisatinic acid* (XVI). A base-catalysed isomerization of neoanisatin (II) takes place to give neoanisatinic acid (XVI), as in the case of anisatin (I). Comparison of the NMR spectra of II and XVI reveals that a similar isomerization has occurred. The signal of the C-8 proton disappears and the methylene signal of C-12 moves to higher field by ca. 2 ppm (4·56 ppm (CF_3COOH) in II; 2·57 ppm (CF_3COOH)



II XVI

in XVI) by transformation of II to XVI. The structure XVI was assigned to neoanisatinic acid. Methylation of XVI with ethereal diazomethane affords methyl neoanisatinate (XVII).

On heating with acetic anhydride and pyridine, neoanisatinic acid (XVI) yields a neutral compound, neoanisatinic acid anhydroacetate (XVIII). In the IR spectrum, XVIII shows no acetate carbonyl band, whereas in the NMR spectrum a new Me signal appears at 1·73 ppm (s) in addition to the two Me signals at 1·12 ppm (d, J = 6·5 c/s) and 1·37 ppm (s) arising from Me groups on C-1 and C-5. This compound, XVIII, was deduced to have an ortho acetate structure corresponding to VIIa obtained from anisatinic acid (III).

Thermal isomerization

4 *Properties of isoanisatinic acid* (XIX). When crystals of anisatin (I) are heated at ca. 300° under nitrogen, exhaustive decomposition affords carbon dioxide and diacetyl as volatile components. Purification of a residual dark-brown solid was difficult, owing to poor solubility in common organic solvents. In contrast, on keeping anisatin (I) at temperatures slightly higher than the m.p. for short periods,

isomerization takes place to isoanisatinic acid (XIX), the yield of XIX depending on temperature and time of heating. A sodium salt of XIX, obtained by treating with a sodium bicarbonate solution, shows bands at 1740 cm^{-1} (δ-lactone) and ca. 1600 cm^{-1} (carboxylate) in the IR spectrum; whereas a sodium salt of XIX, obtained by heating in a sodium hydroxide solution, shows only an intense band at 1590 cm^{-1}. The acid XIX may be regenerated from both sodium salts upon acidification with dilute hydrochloric acid.

The NMR spectrum (in trifluoroacetic acid) of XIX exhibits a secondary Me (1·02 ppm, d, J = 7·0 c/s) and a tertiary Me (1·85 ppm, s). Furthermore, signals corresponding to five protons appear in a complex pattern in the range of 1·6–3·0 ppm. A quartet of the —CH_2—O— type centered at 4·45 ppm (2H, AB-type, J = 14·0 c/s), a singlet at 4·65 ppm (1H), a multiplet* at ca. 4·7 ppm (1H) and a triplet at 4·92 ppm (1H, J = 4·0 c/s) appears in the region of 4–5 ppm. Methylation of XIX with diazomethane gives methyl isoanisatinate (XX), which on acetylation with acetic anhydride and pyridine yields methyl isoanisatinate diacetate (XXI). Compound XXI contains one OH group since a singlet at 5·00 ppm (1H) in the NMR spectrum disappears on addition of deuterium oxide (cf. Fig. 2).

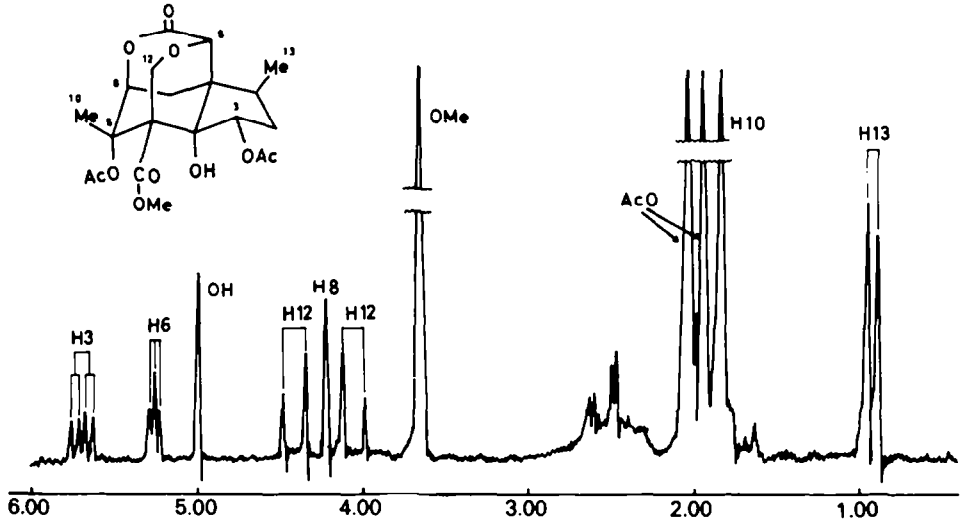
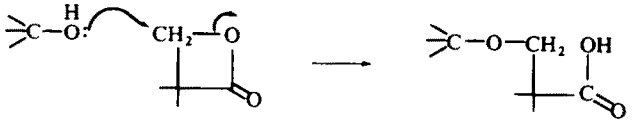


FIG. 2. The NMR spectrum of methyl isoanisatinate diacetate (XXI): ppm from internal TMS at 100 Mc in deuteriochloroform.

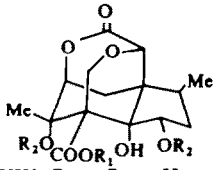
5 *Structure of isoanisatinic acid* (XIX). The IR spectra of the sodium salts of XIX and pKa' measurements show that XIX is a lactone–carboxylic acid. Of the two carbonyl bands (ν_{max}^{Chf} 1826, 1739 cm^{-1}) in anisatin (I), a band due to the β-lactone is absent in XIX. A δ-lactone of I must be intact in XIX, because the sodium salt of XIX prepared by treatment with a sodium bicarbonate solution shows a band at 1740 cm^{-1} (δ-lactone). The carboxyl group in XIX must be derived from the β-lactone of I. The NMR spectrum (Fig. 2) of the diacetate XXI, reveals that isoanisatinic acid (XIX) contains one carboxyl group and three OH groups. A comparison of the

* The shape of this signal was not clear, because of the overlapping with the signal at 4·65 ppm.

functional groups of I and XIX shows that during thermal isomerization a OH group is lost with concomitant formation of a carboxyl group. The isomerization is satisfactorily accounted for by the following intramolecular reaction.



A secondary OH group alpha to a δ-lactone in I is situated behind the methylene group of a β-lactone. The OH group occupying a sterically favourable position must be involved in the isomerization, as depicted in the formulae above. The structure of isoanisatinic acid was thus deduced to be XIX, and confirmed by the NMR spectrum of XXI shown in Fig. 2. The NMR spectrum (Fig. 2) of XXI corresponds with that[1c, 1e] of anisatin triacetate (XXII), supporting the inference that the isomerization involves a β-lactone moiety and a secondary OH attached to a δ-lactone ring and no other groups.



XIX: $R_1 = R_2 = H$
XX: $R_1 = Me$, $R_2 = H$
XXI: $R_1 = Me$, $R_2 = Ac$

6 *Discussion on the thermal isomerization.* It is of interest to examine the stability of a compound lacking a secondary OH group alpha to a δ-lactone in I under conditions employed in the thermal isomerization of I. Noranisatin (XIII) remains unchanged under the isomerization conditions of I. A β-lactone of XIII is quite stable even at elevated temperatures (230° or more). These facts suggest that a OH group must be present at the back of the methylene of the β-lactone in order to effect thermal isomerization.

EXPERIMENTAL

All m.ps were uncorrected. The IR spectra were recorded with a JASCO IR-S spectrophotometer and with a JASCO DS-402G spectrophotometer. The NMR spectra were recorded with Varian Associates spectrometers, A-60 and HA-100; only prominent peaks are cited; chemical shifts are given in ppm relative to internal TMS; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br., broad; coupling constants are given in c/s. The optical rotations and ORD curves were recorded with a JASCO Model ORD/UV-5 spectrometer. 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. TLC analysis was performed on silica gel G or silica gel GF (E. Merck, A.G., Germany). For column chromatography, Mallinckrodt silicic acid (100 mesh, Mallinckrodt, U.S.A.) was used.

Anisatinic acid (III). The acid III was obtained from anisatin (I) by Lane's procedure[2] or as follows: To an aqueous soln of KOH (0·7 g/7 ml), crystalline I (1·5 g) was added gradually with cooling in an ice-bath and the resulting soln was allowed to stand for 1 hr at room temp and acidified (pH 1–2) with 3N HCl. The soln was extracted with three 15 ml portions of AcOEt. The AcOEt extracts were washed with H_2O and sat NaCl aq and dried over Na_2SO_4. On evaporation of the solvent under reduced press, crystals of III were obtained, 1·02 g. The residue (0·065 g) obtained by evaporation of the mother liquor was chromatographed over silicic acid (4 g) with $CHCl_3$–MeOH (98:2) to give additional III, 0·02 g. Re-

crystallization from acetone gave pure III, m.p. 216–218° (lit.,[2] 218–221°); $[\alpha]_D^{20}$ −27° (*c*, 0·5, dioxan); p*Ka* 3·8 (H_2O); IR bands at 1757, 1725 cm^{-1} (THF), 1765, 1735, 1710 (shoulder) cm^{-1} (KBr); NMR spectrum in CF_3COOH (Fig. 1); NMR signals (CD_3COCD_3) at 1·07 (3H, d, J = 6), 1·34 (3H, s), 4·13 (1H, t, J = 2·5), 4·56 (1H, q, J = 4·0, 9·0), 6·4 (5H, br.s, OH); mass, 328 (M^+), 310, 292, 282. (Found: C, 55·06; H, 6·32. $C_{15}H_{20}O_8$ requires: C, 54·87; H, 6·14%.)

Isomerization of I *to* III *under a mild condition.* To an aqueous soln of K_2CO_3 (50 mg/2 ml), a small amount of crystalline I was added and dissolved completely at room temp after 2 days. The soln was acidified with dil HCl and extracted with AcOEt. By the usual workup crystals were obtained from the AcOEt extract, which were identified as III by IR spectral comparison. A similar experiment employing Na_2CO_3 was carried out to give III.

Action of alkali on III. Crystals of III (ca. 350 mg) were dissolved in $Ba(OH)_2$ aq (1·2 g/40 ml). The soln was heated in a sealed tube at ca. 130° for 42 hr. The brown soln was acidified with dil H_2SO_4 and precipitated $BaSO_4$ was removed by filtration. The filtrate was concentrated under reduced press. The residue was dissolved in $CHCl_3$ and chromatographed over silicic acid. Fractions eluted with $CHCl_3$ contained a small amount of yellow material. From the fractions eluted by $CHCl_3$–AcOEt (2:1) crystalline III was obtained.

Action of acid to III. (a) Crystals of III were dissolved in HI with a small amount of red P. The mixture was heated in a water-bath for ca. 20 hr, diluted with H_2O and extracted with AcOEt. From the extract crystalline III was recovered unchanged. (b) An aqueous soln of III containing a small amount of *p*-TsOH was refluxed for 8 hr. By the usual workup crystals of III were recovered.

Methyl anisatinate (IV). The methyl ester, IV, was prepared by Lane's procedure.[2] Recrystallization from acetone afforded pure IV, m.p. 227–228° (lit.[2] 230–232°); IR bands at 1745, 1715 cm^{-1} (in $CHCl_3$); 1763, 1730 cm^{-1} (in THF); mass 342 (M^+), 314, 306, 293, 292. (Found: C, 56·22; H, 6·64. $C_{16}H_{22}O_8$ requires: C, 56·13; H, 6·48%.)

Action of HIO_4 *on* III *and* IV. Samples (10–20 mg) were allowed to react with excess HIO_4 in H_2O (in case of III) or in 30% H_2O–MeOH (in case of IV) for varying lengths of time, after which KI was added and the liberated I_2 titrated with $Na_2S_2O_3$ aq (temp 16°).

III: 0·88 mole (10 min), 0·93 mole (25 min), 0·98 mole (80 min), 1·02 moles (3 hr), 1·07 moles (4 hr).
IV: 0·95 mole (10 min), 1·15 moles (30 min), 1·21 moles (80 min), 1·23 moles (5 hr).

To a soln of III (330 mg) in H_2O (15 ml) a soln of $HIO_4 \cdot 2H_2O$ (230 mg) in H_2O (10 ml) was added and the mixture kept at room temp for 1·5 hr and extracted with four 15 ml portions of AcOEt. The combined AcOEt extracts were washed with H_2O, sat NaCl aq and dried over Na_2SO_4. On evaporation of the solvent a colourless oily material (330 mg) was obtained and chromatographed over silicic acid (15 g). Fractions eluted with $CHCl_3$–MeOH (5%) gave colourless resin (280 mg), which did not crystallize.

Methyl anisatinate carbonate (V). Phosgene gas was passed through a soln of IV (300 mg) in THF (15 ml) and pyridine (0·5 ml) under cooling. The soln became turbid. After ca. 20 min a solid deposited and the soln was transparent. For further 20 min phosgene gas was passed through the mixture. After removal of the solvent, water was added to the residue oil, and the resulting crystals filtered off and dried, 220 mg. Recrystallization from EtOH afforded pure V, m.p. 200–203° (sublimation); IR bands at 1805, 1750, 1725 cm^{-1} (KBr); NMR signals (CD_3COCD_3) at 1·19 (3H, d, J = 6), 1·40 (3H, s), 3·78 (3H, s), 4·15 (1H, s, OH), 4·27 (1H, t, J = 2·5), 4·73 (1H, s, OH), 5·23 (1H, d, J = 7); mass, 368 (M^+), 353, 337, 325, 306, 307. (Found: C, 55·49; H, 5·43. $C_{17}H_{20}O_9$ requires: C, 55·43; H, 5·47%.)

Methyl anisatinate diacetate (VI). A soln of IV (600 mg) in Ac_2O (8 ml) and pyridine (2 ml) was kept at room temp for 3 days and subsequently at 95° for 5 hr. The soln was concentrated under reduced press to give a yellow oil, which on addition of H_2O (5 ml) and standing gradually crystallized. The solid was were dried and recrystallized from $CHCl_3$–benzene to give needles of VI, 580 mg, m.p. 187–188°; IR bands at 1765, 1735 (strong) cm^{-1} (KBr); NMR signals ($CDCl_3$) at 1·07 (3H, d, J = 6), 1·73 (3H, s), 2·01 (3H, s), 2·07 (3H, s), 3·48 (1H, s, OH), 3·70 (3H, s), 3·90 (1H, s, OH), 5·11 (1H, t, J = 3), 5·70 (1H, q, J = 6, 9); mass, 426 (M^+), 408, 395, 384, 383, 366. (Found: C, 56·60; H, 6·13. $C_{20}H_{26}O_{10}$ requires: C, 56·33; H, 6·15%.)

Anisatinic acid anhydrodiacetate (VII). A soln of III (1 g) in Ac_2O (12 ml) and pyridine (3 ml) was kept at room temp for 3 days and then at 90–95° for 4 hr. The mixture was concentrated under reduced press to give a yellow oil which on addition of H_2O (5 ml) and standing gradually crystallized. The solid was filtered off, dried and recrystallized from $CHCl_3$–CCl_4 (3 times), 500 mg, m.p. 199–201°; IR bands at 1795, 1765, 1730 cm^{-1} (KBr); NMR signals ($CDCl_3$) at 1·13 (3H, d, J = 6), 1·39 (3H, s), 1·81 (3H, s), 2·08 (3H, s), 3·65 (1H, s, OH), 4·26 (1H, t, J = 3), 5·43 (1H, q, J = 4, 9); mass, 394 (M^+), 366, 352, 350, 334. (Found: C, 58·00; H, 5·53. $C_{19}H_{22}O_9$ requires: C, 57·86; H, 5·62%.)

Anisatinic acid acetate (VIII). A soln of I (130 mg) in AcOH (5 ml) containing *p*-TsOH (15 mg) was refluxed for 18 hr and concentrated under reduced press to afford an oil which after addition of water was extracted with three 15 ml portions of AcOEt. The combined extracts were washed with H_2O and sat NaCl aq and dried over Na_2SO_4. On removal of the solvent, crystals were obtained. Recrystallization from $CHCl_3$–benzene afforded fine needles of VIII, 110 mg, m.p. 240–242° (sublimation); IR bands at 1765, 1748, 1690 cm^{-1} (KBr); NMR signals (CD_3COCD_3) at 1·12 (3H, d, J = 6·5), 1·33 (3H, s), 2·04 (3H, s, —$OCOCH_3$), 4·13 (1H, t, J = 3), ca. 4·8 (4H, br. s, OH), 5·70 (1H, q, J = 5, 9·5); mass, 370 (M^+), 352, 334, 310, 309. (Found: C, 55·50; H, 5·94. $C_{17}H_{22}O_9$ requires: C, 55·13; H, 5·99%.)

Anisatinic acid formate (IX). A soln of III (100 mg) in HCOOH (5 ml) was heated under reflux for 14 hr. The colourless transparent soln was concentrated under reduced press to give white crystals, which were recrystallized from AcOEt, 82 mg, m.p. ca. 245° (sublimation); IR bands at 1745, 1720 (strong) cm^{-1} (KBr); mass, 356 (M^+), 338, 320, 310, 290, 274. (Found: C, 54·00; H, 5·41. $C_{16}H_{20}O_9$ requires: C, 53·93; H, 5·66%.)

Methyl anisatinate ketone (X). To a soln of IV (500 mg) in AcOH (20 ml) an aqueous soln (10 ml) of $KMnO_4$ (450 mg) was added with stirring at room temp. The mixture was allowed to stand at room temp for 2 days and evaporated under reduced press below 50°. To the residue H_2O was added and the mixture extracted with AcOEt. The extract was washed with H_2O and sat HaCl aq and dried over Na_2SO_4. Evaporation of the solvent under reduced press yielded a brown oily product (ca. 260 mg), which was chromatographed on silicic acid. From the fractions eluted with $CHCl_3$–AcOEt (3:1) crystals (ca. 30 mg) were obtained. Recrystallization from $CHCl_3$–AcOEt afforded pure X, m.p. ca. 210° (sublimation); IR bands at 1750, 1725 cm^{-1} (KBr); mass, 340 (M^+), 322, 309, 291, 270. (Found: C, 56·28; H, 5·96. $C_{16}H_{20}O_8$ requires: C, 56·46; H, 5·92%.)

Anhydroanisatic acid (XII). To crystals of XI (200 mg), 0·1N NaOH aq (8 ml) was added dropwise. The mixture was allowed to stand overnight below 0° and then 0·1N NaOH aq (1 ml) was added. The soln, after 1 hr was acidified with 1N HCl and extracted with AcOEt. The AcOEt layer was further extracted with $NaHCO_3$ aq. The $NaHCO_3$ soln was acidified with HCl and extracted with AcOEt. The AcOEt extract was washed with sat NaCl aq and dried over Na_2SO_4. Evaporation of the solvent, yielded an oil (157 mg), which was chromatographed over silicic acid. Fractions eluted with AcOEt–hexane (1:1) afforded crude crystals, which on recrystallization from AcOEt gave XII, 20 mg, m.p. 157–158°; IR bands at 1725 (shoulder), 1710 cm^{-1} (KBr); mass, 328 (M^+), 310, 300, 292, 279, 266. (Found: C, 54·82; H, 6·13. $C_{15}H_{20}O_8$ requires: C, 54·87; H, 6·14%.)

Neoanisatinic acid (XVI). Compound II (150 mg) was dissolved in an aqueous soln (8 ml) of $Ba(OH)_2 \cdot 8H_2O$ (160 mg) at room temp. The soln was allowed to stand overnight at room temp and acidified with dil H_2SO_4. The precipitated $BaSO_4$ was filtered off and the filtrate was concentrated under reduced press to give white crystals. Recrystallization from AcOEt–$CHCl_3$ afforded pure XVI, 110 mg, m.p. 229–231°; pKa' 3·8 (H_2O); $[\alpha]_D^{20}$ −51° (c, 0·7, dioxan); IR bands at 1765, 1728 cm^{-1} (KBr); NMR signals (CF_3COOH) at 1·23 (3H, d, J = 6·0), 1·65 (3H, s), 4·52 (1H, t, J = 2·5); mass, 312 (M^+), 294, 276, 266, 258, 248. (Found: C, 57·75; H, 6·59. $C_{15}H_{20}O_7$ requires: C, 57·68; H, 6·46%.)

Methyl neoanisatinate (XVII). Crystals of XVI (400 mg) were added to a soln of ethereal diazomethan. Evaporation of the resulting soln gave the crude ester, which was recrystallized from CCl_4–benzene to afford XVII, 320 mg, m.p. 185–186°; IR bands at 1748, 1728 cm^{-1} (THF); NMR signals ($CDCl_3$) at 1·10 (3H, d, J = 6·5), 1·40 (3H, s), 3·55 (1H, s, OH), 3·83 (3H, s, —COOMe), 4·25 (1H, t, J = 3·0), ca. 4·4 (1H, br. s, OH), ca. 5·2 (1H, br. s, OH); mass, 326 (M^+), 308, 290, 280, 276. (Found: C, 58·98; H, 6·84. $C_{16}H_{22}O_7$ requires: C, 58·88; H, 6·80%.)

Neoanisatinic acid anhydroacetate (XVIII). A soln of XVI (1 g) in pyridine (3 ml) and Ac_2O (10 ml) was kept at room temp for 3 days and heated at ca. 90° for 4 hr. The mixture was concentrated under reduced press to give an oil to which H_2O was added. After 2 hr, H_2O was removed by decantation and the residue after drying in a desiccator was chromatographed over silicic acid with $CHCl_3$. Early fractions afforded crude XVIII, which on recrystallization from CCl_4 gave pure XVIII, m.p. 153–154°; IR bands at 1790, 1760 cm^{-1} (KBr); NMR signals ($CDCl_3$) at 1·12 (3H, d, J = 6·5), 1·37 (3H, s), 1·73 (3H, s), 3·63 (1H, s, OH), 4·25 (1H, t, J = 3·0); mass, 336 (M^+), 308, 292, 276. (Found: C, 60·50; H, 6·22. $C_{17}H_{20}O_7$ requires: C, 60·71; H, 5·99%.)

Pyrolysis of I. Crystals of I (1·9 g) in a flask were heated at ca. 300° in a metal bath under N_2 for 15 min. The gas generated was led to a 2N HCl soln of 2,4-dinitrophenylhydrazine by a stream of N_2. The resulting crude 2,4-dinitrophenylhydrazone was filtered off, dried and washed with MeOH–benzene (1:4), 120 mg. Repeated recrystallization from nitrobenzene afforded needles, 60 mg, m.p. 304–307° (dec).

(Found: C, 43·27; H, 2·99; N, 24·78. $C_{16}H_{14}O_8N_8$ requires: C, 43·06; H, 3·16; N, 25·10%.) This 2,4-dinitrophenylhydrazone was identified as bis-2,4-dinitrophenylhydrazone of diacetyl by IR spectral comparison with an authentic sample. The dark-brown residue in the flask was almost insoluble in AcOEt and sparingly soluble in $CHCl_3$. The residue soluble in $CHCl_3$ was chromatographed on silicic acid to give a coloured amorphous material. Pyrolysis of I was repeated and CO_2 gas was introduced to a $Ba(OH)_2$ soln. From the amount of precipitated $BaCO_3$, it was shown that ca. 0·8 mole of CO_2 was obtained.

Isoanisatinic acid (XIX). Under N_2 atmosphere, crystals of I (990 mg) in a flask were heated in a metal bath to 227–230° for 3 min. After cooling the resulting brown solid was dissolved in $CHCl_3$ (30 ml). The $CHCl_3$ soln was extracted with three 30 ml portions of sat $NaHCO_3$aq. The combined aqueous layers were acidified with 3N HCl to pH 2 and extracted with three 30 ml portions of AcOEt. The AcOEt extracts were washed with H_2O and sat NaClaq, dried over Na_2SO_4 and evaporated under reduced press to give a brown solid (727 mg). Chromatography of the crude products over silicic acid (35 g) with $CHCl_3$–MeOH (97:3) afforded crude crystals (279 mg) and an oily material (329 mg). The former was recrystallized from $CHCl_3$–AcOEt to give needles, 243 mg; recrystallization was repeated three times, m.p. 242–247°; $[\alpha]_D^{25}$ −73° (*c*, 0·7, dioxan); p*Ka'* 3·9 (H_2O); IR bands at 1730 (br., strong) cm^{-1} (KBr); NMR signals (CF_3COOH) at 1·02 (3H, d, J = 7·0), 1·85 (3H, s), 1·6–3·0 (5H, complex pattern), 4·45 (2H, q, AB-type, J = 14·0), 4·65 (1H, s), ca. 4·7 (1H, m?), 4·92 (1H, t, J = 4·0); mass, 328 (M^+), 310, 292. (Found: C, 54·89; H, 6·39. $C_{15}H_{20}O_8$ requires: C, 54·87; H, 6·14%.) Without an extraction procedure using $NaHCO_3$aq, the acid XIX was obtained by direct chromatography of the solid produced after heating over silicic acid. The yield of XIX was greatly influenced by conditions employed: at 226–230° for 10 min, 700 mg of I afforded 105 mg of XIX and at 245–250° for 3 min, 1087 mg of I gave only 60 mg of XIX.

Methyl isoanisatinate (XX). A soln of XIX (150 mg) in THF (3 ml) was added dropwise to an excess of ethereal diazomethane. After 5 hr the soln was concentrated and the resulting solid was recrystallized from $CHCl_3$–benzene, 125 mg, m.p. 207–209°; IR bands at 1740, 1720 (shoulder) cm^{-1} (KBr); mass, 342 (M^+), 324, 306, 293, 292, 282. (Found: C, 55·90; H, 6·52. $C_{16}H_{22}O_8$ requires: C, 56·13; H, 6·48%.)

Methyl isoanisatinate diacetate (XXI). A soln of XX (91 mg) in Ac_2O (0·5 ml) and pyridine (0·8 ml) was kept at 50° for 92 hr and concentrated under reduced press. Water was added to the residue and the resulting mixture was extracted with ether. When the soln was concentrated, crude crystals of XXI were precipitated and filtered off (40 mg). Further concentration of the filtrate afforded 11 mg of XXI. Recrystallization from $CHCl_3$–ether afforded pure XXI, m.p. 149–151°; IR bands at 1730, 1760 (shoulder) cm^{-1} (KBr); NMR spectrum (Fig. 2); mass, 426 (M^+), 408, 396, 395, 384, 366. (Found: C, 55·83; H, 6·22. $C_{20}H_{26}O_{10}$ requires: C, 56·33; H, 6·15%.)

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