Tetrahedron. Vol. 25, pp. 1323 to 1334. Pergamon Press 1969. Printed in Great Britain

SOME NEW TERPENOID METABOLITES FROM AN UNIDENTIFIED FUSARIUM SPECIES

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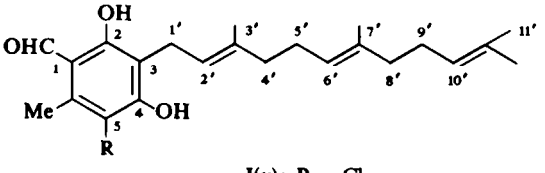
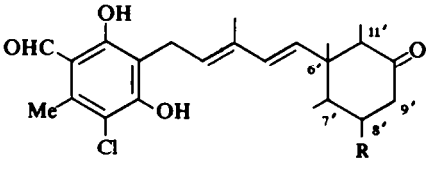
(*Received in USA* 19 *June* 1968; *Received in the UK for publication* 18 *October* 1968)

Abstract—Six new terpenoids of mixed biogenetic origin exhibiting anti-*Tetrahymena pyriformis* activity have been isolated from an unclassified Fusarium species designated LL-Z1272. The structures of these metabolites, named LL-Z1272α, β, γ, δ, ε and ζ, are composed of an orcyl aldehyde unit condensed with a farnesyl side chain which in γ, δ, ε and ζ is terminally cyclized to a cyclohexanone ring. All but β and ε contain a chlorine atom in the aromatic portion of the molecule.

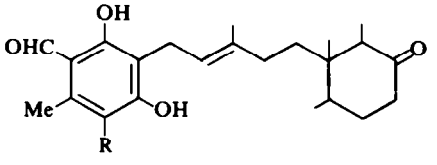
IN A continuing search for microbial metabolites with useful biological activity, we have obtained six biogenetically related compounds which inhibit the growth of the protozoan, *Tetrahymena pyriformis*.[1] We wish to describe here work which has led to the structures of these metabolites, called LL-Z1272α, β, γ, δ, ε and ζ (designated throughout the text by I through VI, respectively) which, as seen, consist of an orcyl aldehyde unit condensed with a farnesyl side chain in varying degrees of oxidation. Orsellinic acid was also isolated from fermentation extracts along with the above metabolites.



I(α): R = Cl
II(β): R = H



III(γ): R = H
VI(ζ): R = OAc



IV(δ): R = Cl
V(ε): R = H

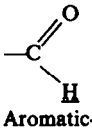
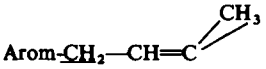
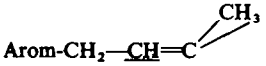
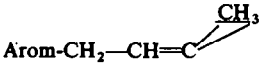
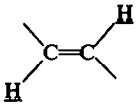
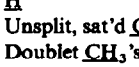
The orcyl aldehyde portion of the metabolites was suggested by their UV spectra in both neutral and basic solution (Table 1). The 235 mμ region of the spectra of III and VI is complicated somewhat due to the superposition of the diene and the aromatic chromophores although the E. T. bands were essentially identical with the other metabolites. The purple-brown colour with aqueous ferric chloride was also strongly reminiscent of the orcyl aldehyde system as were the IR and NMR spectral properties.

TABLE 1. PRINCIPAL E. T.[2a] BANDS IN ALCOHOLIC AND BASIC SOLVENTS OF THE LL-Z1272 METABOLITES AND SOME SUBSTITUTED BENZALDEHYDES (ACTUAL AND CALCULATED)

Compound	Principal E.T. band (alcohol)	Principal E.T. band (base)	Shift
β-Resorcyl aldehyde[2a]	278 mμ	331 mμ	53
Orcyl aldehyde	289	337	48
(I)	293	348	55
(II)	297	341	44
(III)	292	248	56
(IV)	293	348	55
(V)	295	342	47
(VI)	293	348	55
3,6-Dialkyl-β-resorcyl aldehyde (Calc'd)[2b]	288	345	57
3,6-Dialkyl-5-chloro-β-resorcyl aldehyde (Calc'd)[2b]	288	345	57

The IR spectra of all six components are characterized by strong carbonyl absorption around 1630 cm^{-1} in accord with a 2,4-dihydroxy benzaldehyde system[3a] and the NMR spectra of all the metabolites exhibit an aldehydic proton signal at 600–607 Hz and the chelated phenolic hydrogen signal at 757–761 Hz (Table 2). The remaining para phenolic hydroxyl signal was observed at 389–405 Hz by exchange with CD_3OD. Although this latter signal was somewhat obscured in the spectrum of V,

TABLE 2. NMR VALUES OF VARIOUS FUNCTIONAL GROUPS IN I–VI ABSTRACTED FROM THEIR SPECTRA

Functionality	NMR values (Hz relative to TMS)					
	I	II	III	IV	V	VI
Chelated O<u>H</u>	759	759	759	757	758	761
Non-chelated-O<u>H</u>	389	396	405	399	396	400
	605	600	606	604	600	607
Aromatic-C<u>H</u>$_3$	153	148	154	155	146	155
Aromatic-<u>H</u>	—	372	—	—	376	—
	203	203	211	203	196	212
	315	316	332	315	315	334
	108	108	116	108	108	116
	—	—	324	—	—	322
	—	—	354	—	—	355
Unsplit, sat'd C<u>H</u>$_3$	—	—	42	33	32	44
Doublet C<u>H</u>$_3$'s[2]	—	—	49, 50	52, 53	51, 51	53, 53

the metabolite readily formed a mono-O-Me ether and a phenolic monoacetate (1760 cm^{-1}),[3b] both of which still contain the chelated carbonyl system. The 3-proton singlet at 146–155 Hz observed in all the metabolites was assigned to the aromatic Me group which is considerably deshielded by the adjacent carbonyl.

Table 3 compares the chemical shifts of aromatic Me hydrogens in varying environments, and, as can be seen, those for the LL-Z1272 metabolites compare favorably with that for the Me signal in orcyl aldehyde.

TABLE 3. NMR VALUES OF AROMATIC Me HYDROGENS IN VARYING ENVIRONMENTS

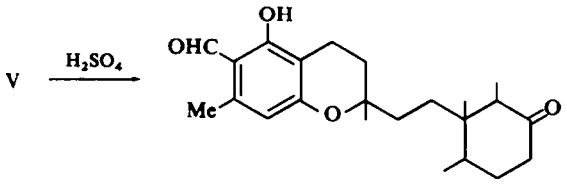
Compounds	NMR value (Hz) of aromatic Me hydrogens relative to TMS
o-Cresol	137[4]
m-Cresol	136[4]
p-Cresol	138[4]
Orcinol	130
Methyl pyrogallol	118
Orcyl aldehyde	148
I	153
II	148
III	154
IV	155
V	146
VI	155

I, III, IV and VI contain chlorine and have no resonances in their NMR spectra corresponding to aromatic hydrogens whereas the two metabolites without chlorine, II and V, each exhibit a single aromatic proton signal (one-proton singlet) at 372 and 376 Hz, respectively (Table 2). This suggests that in I, III, IV and VI the chlorine is on the aromatic ring, replacing the aromatic hydrogen seen in II and V.

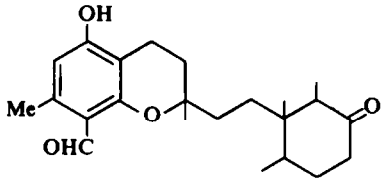
It remained to decide as to the position of the chlorine and the terpenoid side chain. Evidence that the chlorine, when present, is located at C-5 could be obtained by the fact that the aromatic Me group in all the chlorine-containing metabolites experiences an additional deshielding (~7 Hz) in contrast with the two which lack chlorine (Table 3), consistent with an electronegative chlorine atom being ortho to the Me group. The UV spectra of the four chlorine-containing metabolites show an appreciable peak at 345–347 mμ even in neutral or acidic conditions suggesting the chlorine through hydrogen bonding, is aiding in the partial ionization of the chromophore. The UV spectra of the two metabolites devoid of chlorine lack this absorption under the same conditions.

Chemical evidence that the terpenoid side chain is attached at C-3 as opposed to the C-5 position of the orcyl aldehyde moiety was obtained by treatment of V with concentrated sulfuric acid which yielded the two chromanes (VII and VIII) after chromatography over silica gel. The NMR spectra of these two chromanes were identical except that the spectrum of VII lacked the C-4 phenolic OH signal but

retained the signal of the chelated OH proton. This situation was reversed in the spectrum of VIII.

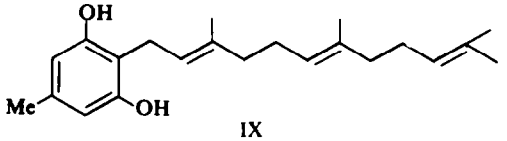


VII



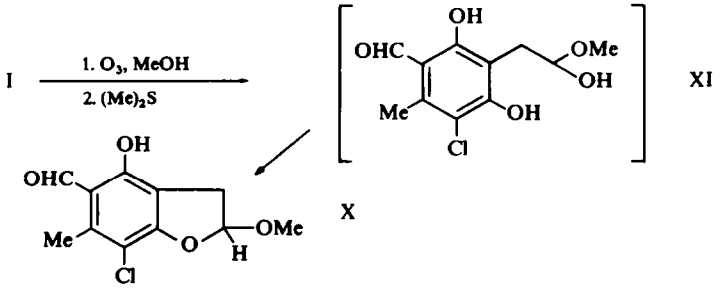
VIII

Aside from the fact that I contained chlorine and II did not, their spectral data suggested they were identical. Their NMR spectra were essentially the same except for the difference in chemical shift for the appropriate hydrogens and that the spectrum of II contained one aromatic proton resonance at 372 Hz (one-proton singlet) whereas the spectrum of I did not. The non-aromatic portions of their spectra, which cover the residual $C_{15}H_{25}$ portion of each molecule, suggested the presence of a farnesyl moiety. Indeed, comparison of the NMR spectra of I and II with that of grifolin (IX)[5]



IX

in conjunction with stereochemical assignments for the four isomeric farnesols based on their NMR spectra[6] gave additional support for this view, and, in addition, suggested a *trans-trans* farnesyl grouping. Ozonolysis of I gave levulinic aldehyde and acetone, isolated as their 2,4-DNP's in a 2:1 ratio and also provided the aromatic portion of the molecule as X. This latter fragment most likely arises from the species XI during the workup (Experimental). Various attempts to decarbonylate II to give

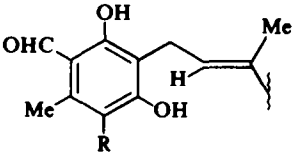


XI

X

grifolin including the method of Tsuji and Ohno[7] with chlorotris (triphenylphosphine) rhodium were not successful.

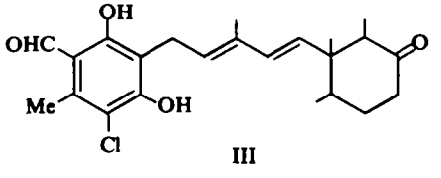
Subtraction of the orcyl aldehyde portion from the molecular formulas of III, IV and V indicated these metabolites have side chains of $C_{15}H_{23}O$, $C_{15}H_{25}O$ and $C_{15}H_{25}O$, respectively. The oxygen atom was shown to be present as a ketone by IR absorption at 1706–1709 cm^{-1} and by the lack of additional aldehydic proton or methyl ketone signals in their NMR spectra. Aside from the ketone, there are three additional degrees of unsaturation to be accounted for in III and two each in IV and V. One of these is accounted for by a trisubstituted double bond as evidenced by the presence in the NMR spectra of resonances corresponding to the C-1′ methylene group, the C-2′ vinyl proton and the C-3′ vinyl Me (Table 2) of I and II. Hence, the partial structure XII can be written for these three compounds.



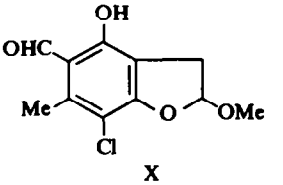
XII

Of the remaining two degrees of unsaturation in III, one can be accounted for by a *trans*-disubstituted double bond which is conjugated with the above-mentioned trisubstituted double bond as shown by the NMR and UV spectra. A sharp four-line AB pattern at 324 and 354 Hz (J = 16 Hz) indicates a *trans*-orientation of the two protons and the lack of additional coupling indicates the adjacent carbon atoms are devoid of protons. A very high maximum at 238 mμ (ε ~35,000) in the UV spectrum of III as compared with the spectra of the IV (231 mμ, ε ~20,000) and V (233 mμ, ε ~12,000) components suggests the presence of a diene chromophore.

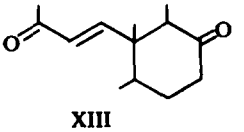
The remaining degree of unsaturation in III can be accounted for either by a tetrasubstituted double bond or a carbocyclic ring (no remaining olefinic protons to be assigned in its NMR spectrum). Besides the previously discussed functional groups, the NMR spectrum of III shows a tertiary C–Me signal at 42 Hz (3-proton singlet) and two secondary C–Me groups at 49 and 50 Hz (3-proton doublets, $J \approx 6{\cdot}0$ Hz). The chemical shifts of these C–Me groups disallow their placement on a double bond, and, with the aforementioned data suggest the presence of a trimethylcyclohexanone moiety. Ozonolysis of III gave X, also obtained from I by similar treatment and the



III



X

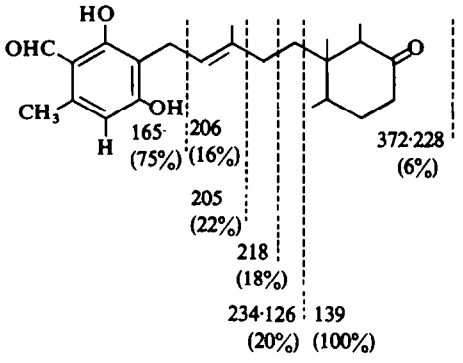


XIII

α,β-unsaturated methyl ketone XIII which together account for all the carbon atoms in III. The discussion of the substitution pattern of the cyclohexanone ring will be deferred until later.

Other than lacking the *trans*-disubstituted double bond and having the additional methylene hydrogens, the NMR spectrum of IV was very similar to that of III. The principal difference between the two spectra, other than that just mentioned, was that the signals in the spectrum of IV for the tertiary C-Me group and the methyl, methylene and proton attached to the tri-substituted double bond are found upfield from the corresponding signals in the NMR spectrum of III. These differences are consistent with IV being the dihydro derivative of III and chemical support was provided by reduction of both compounds (H_2, 10% Pd/Ca CO_3) to give identical oily products (TLC, IR, UV, NMR).

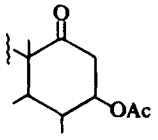
Other than the already mentioned difference in the aromatic portion of their molecules, the NMR spectra of IV and V indicated them to be identical. This implies V to be the dechloro derivative of IV. The mass spectrum of V is in accord with the structure formulated for it, having major fragmentation ions corresponding to cleavage at the more likely positions in the side chain as is shown below.



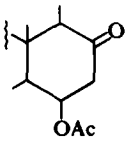
By comparison of their spectra, VI and III appear to be very closely related. In their NMR spectra, particularly, the hydrogen signals corresponding to the aromatic portion and the tri- and *trans*-disubstituted double bonds of VI are the same as for III. Also, the NMR spectrum for VI shows the two Me doublets and one Me singlet as does that for III, and the IR spectrum of VI has a normal ketone band (1715 cm^{-1}) similar to III. Examination of their molecular formulas, however, reveal that VI ($C_{25}H_{31}O_6Cl$) contains the elements $C_2H_2O_2$ more than III ($C_{23}H_{29}O_4Cl$), which could be ascribed to the presence of an acetoxy group. This is supported in the IR spectrum of VI since, in addition to the ketone band, it has bands at about 1740 and 1240 cm^{-1} which are characteristic of the acetate function, and are lost after mild base treatment of VI concurrent with a shift in the ketone band from 1715 to 1675 cm^{-1}. This can best be explained by elimination of the acetoxy grouping β to the carbonyl group to give an α,β-unsaturated ketone.

A closer examination of the NMR spectra of VI, and of its base product suggests the arrangement of the various substituents of the cyclohexanone ring. The NMR spectrum of VI shows one hydrogen (294 Hz) on the carbon having the acetoxy group,

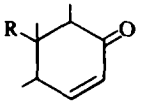
which appears to be the X-hydrogen of an AMNX system, being coupled to three other hydrogens ($J \approx 6{\cdot}0$, 9·5 and 10·5 Hz). Although only one of them can be seen clearly, the two hydrogens representing the MN portion of the system (144 and 171 Hz), in addition to being coupled to the X-hydrogen by about 10·5 and 6·0 Hz, respectively, are coupled with each other by about 14·0 Hz, suggesting them to be geminal hydrogens. Their chemical shifts in conjunction with their J values indicate them to be the axial and equatorial hydrogens, respectively, on a carbon adjacent to the carbonyl group in a cyclohexanone ring. Hence, two possible substitution patterns can be written for the cyclohexanone ring, XIV and XV.



XIV



XV

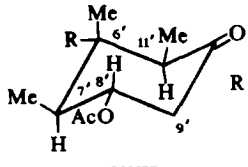


R = Rest of molecule

XVI

The NMR spectrum of the base product (XVI) of VI clearly shows XV to be the correct substitution pattern. As would be expected for either case, the α,β-unsaturated ketone produced by β-elimination of the acetoxy group, shows two *cis*-vinyl proton signals (396 and 356 Hz) coupled with each other (J = 10 Hz) and also with a third hydrogen (J = 2·0 and 3·0 Hz), respectively). The geminal proton resonances are no longer present but in their absence a third proton signal is clearly seen at 148 Hz which appears as a four-line pattern coupled only with the hydrogens of a Me group ($J \approx 7{\cdot}0$ Hz). This clearly indicates XV to be correct since in XIV no third proton on a carbon adjacent to a carbonyl group exists.

Examination of the coupling constants and the chemical shifts for the various hydrogens on the ring in the NMR spectra of VI and its base product indicates the relative stereochemistry of the ring to be as shown in XVII. The C-6′ Me group is most certainly axial due to the bulk of the remainder of the molecule attached at C-6′. The coupling constants between the C-8′ proton and the C-9′ equatorial and axial protons ($J \approx 6{\cdot}0$ and 10·5 Hz, respectively) indicate the C-8′ hydrogen to be axial. The spin–spin coupling between the C-7′ and C-8′ hydrogens ($J \approx 9{\cdot}5$ Hz) suggests the C-7′ hydrogen is also axial. Although the C-11′ hydrogen is insulated from other ring hydrogens, its chemical shift, and that of the C-11′ Me group are the same for both VI and its base product, indicating the C-11′ Me to be in the more stable equatorial position.



R = Rest of molecule

XVII

Because of the similarity in the NMR spectra of III, IV, V and VI of the hydrogens related to the cyclohexanone ring, except for the predictable variations in chemical

shifts due to differing functional groups (OAc in VI; *trans*-disubstituted double bond in III and VI), it is probable that the four components have a common substitution pattern, which is as shown in XVII (—H instead of —OAc for III, IV and V). As mentioned earlier, due to the bulk of the side chain attached at C-6′, the C-6′ Me group can be assigned with reasonable certainty to the axial conformation. Table 4 lists the chemical shifts for Me groups at various positions in a cyclohexanone ring and as can be seen, the 3-axial, stands out with a chemical shift appreciably upfield from the others. The values for the singlet Me in the LL-Z1272 components, 32–44 Hz (Table 2), most nearly agree with the 3-axial value, which is in accord with the position of this Me, relative to the ketone, previously deduced by independent means for the VI component.

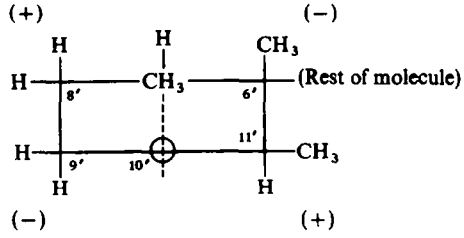
Anet[8] has found that in the NMR spectrum of 3-methylcyclohexanone, the Me group gives a very poor doublet, presumably as a result of the carbonyl group shifting the resonance of the C-2 methylene protons so that they are only slightly separated from the C-3 hydrogen chemical shift. Relating this to the present case of the LL-Z1272 metabolites, it is observed that the two Me doublets present in the spectra of III, IV, V and VI are quite sharp and hence, it can be inferred that neither of these is on the remaining 3-position relative to the ketone, in the cyclohexanone ring. Thus they must be on the 4- and/or 2-positions relative to the ketone.

TABLE 4. NMR VALUES FOR Me GROUPS ON VARIOUS POSITIONS OF A CYCLOHEXANONE RING

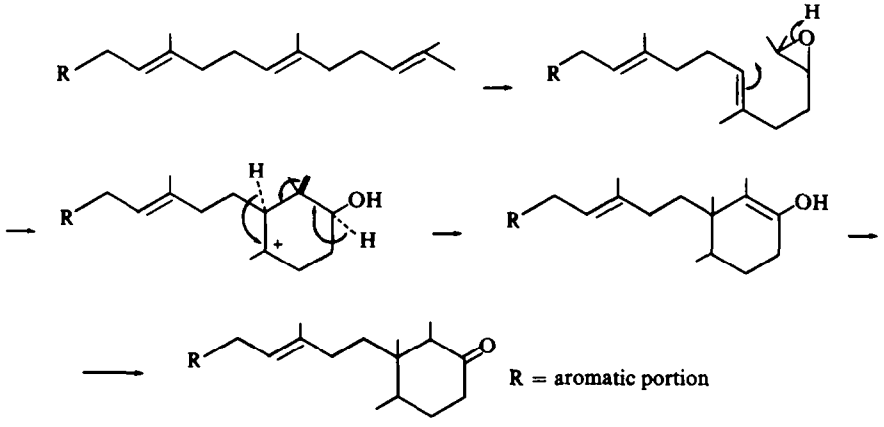
Compound	Position relative to carbonyl (= 1)	Conformation	Chemical shift (Hz relative to TMS = 0)
Dihydrotestosterone acetate[9a]	4	a	62
3α-Acetoxy-12-ketocholanic acid methyl ester[9b]	2	a	62
Androstan-11-one[9c]	3	a	40
trans-4-t-Butyl-2-methylcyclohexanone[10]	2	a	69
cis-4-t-Butyl-2-methylcyclohexanone[10]	2	e	61
trans-2-t-Butyl-4-methyl cyclohexanone[10]	4	a	67
cis-2-t-Butyl-4-methylcyclohexanone[10]	4	e	60
3-Methylcyclohexanone[8]	3	e	61

Johnson *et al.*[10] have found that 2-equatorial and 4-equatorial Me groups on a cyclohexanone ring have about the same chemical shift in deuterochloroform which is somewhat upfield from the corresponding axial cases, but that the 2-Me groups generally have a larger coupling constant. They further observed that change of solvent from deuterochloroform to pyridine leads to a marked displacement to higher field of all peaks (4-equatorial Me greatest) except the 2-equatorial Me group. When this solvent change was tried on V, one of the methyl doublets (J = 6·8 Hz) remained relatively unchanged whereas the other (J = 6·0 Hz) shifted appreciably upfield (> 10 Hz). Based on analogy with the work of Johnson *et al.*, this would indicate one of the methyl doublets to be 4-equatorial relative to the ketone and the other to be 2-equatorial, which is in accord with what was found for VI by the spin–spin coupling of the appropriate hydrogens.

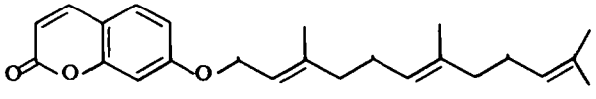
The optical rotatory dispersion curve* determined for V has a large negative cotton effect at 300 mμ which is in agreement with the substitution pattern of the cyclohexanone ring and, in fact, as shown below, indicates its absolute stereochemistry to be as in XVII (—H instead of —OAc).



Although the substitution pattern of the cyclic ketone in III, IV, V and VI is somewhat unusual in terpenoids, its biogenesis is quite consistent with recent studies on the mechanism of terpenoid cyclization[11] and, in addition, re-enforces the stereochemical assignments made above from NMR considerations.



It is interesting to note that appreciable amounts of mono-cyclic products with the LL-Z1272 cyclohexanone substitution pattern were obtained by van Tamelen, Nadeau and Coates from the chemical cyclization of the terminal epoxide of *trans, trans, trans*-geranylgeraniol acetate[12] as well as in the synthesis of the farnesiferols from XVIII.[13]



XVIII

* We thank Dr. J. W. McGahren of these laboratories for this determination and Dr. M. Goodman of the Brooklyn Polytechnic Institute for the use of this instrument (Cary Spectropolarimeter).

EXPERIMENTAL

The IR spectra were taken on a Perkin–Elmer model 137 Infracord. UV spectra were recorded in MeOH. NMR spectra were recorded with a Varian A-60 in $CDCl_3$; shifts are expressed in Hz from TMS as internal standard and coupling constants (J) are also expressed in Hz. M.ps are uncorrected. The mass spectra were obtained in a direct inlet MS9 (AEI). We thank W. J. Fulmor's group for some of the spectral determinations, L. M. Brancone and associates for the elemental analyses, Dr. J. Karliner for the mass spectra, and Dr. P. Shu, A. Shay, M. Dann and associates for the fermentations and preliminary ethyl acetate extractions.

Isolation of the LL-Z1272 *metabolites*

The fermentation mash from a 300 l. tank was twice extracted with ½ volume of AcOEt and the pooled, filtered extracts concentrated to a heavy oil. In order to remove the oil, it was dissolved in CH_2Cl_2 and passed over a 500 g silica gel column. The majority of the compounds were eluted in the first 4 l. of the CH_2Cl_2 effluent, from which over 100 g of crude semicrystalline material was recovered. Continued elution with CH_2Cl_2 led to the isolation of 5 g of crude orsellinic acid which was recrystallized from water to give the pure material m.p. 190–200°. Identification was made by comparison of its physical constants with those given by Bentley *et al.*[14] and Wachtmeister.[15] The crude, crystalline mixture from the above column was chromatographed over silica gel and eluted with hexane followed by a gradient between hexane and CH_2Cl_2, then CH_2Cl_2, and finally a gradient between CH_2Cl_2 and CH_2Cl_2–ether (1:1). The column effluent was continuously monitored at 292 mμ using a Beckman DU spectrophotometer with an attached Brown recorder.

Fraction I (LL–Z1272α). The effluent corresponding to I was concentrated to dryness which provided 14 g of oil which crystallized with difficulty. This material was dissolved in 40 ml each of the upper and lower phases of a MeOH–heptane system (K = 0·9) and placed in the first five tubes of a 10 ml per phase 200-tube solvent countercurrent distribution apparatus. After 353 transfers, tubes numbered 65 to 140 inclusive were pooled and concentrated to a solid. Crystallization from MeOH–isooctane and then MeOH–water provided 6·17 g from the first crop, 0·375 g from the second and 0·470 g from a third; m.p. 72·5–73°. (Found: C, 70·73; H, 8·05; Cl, 9·65. $C_{23}H_{31}O_3Cl$ requires: C, 70·66; H, 7·99; Cl, 9·07%); λ_{max} 228, 293 and 345 mμ (ε 11,150, 10,400 and 7800); $\nu_{max}^{CHCl_3}$ 1629 cm^{-1}.

Fraction II (LL-Z1272β). Fraction II was concentrated to a small volume from which 2·5 g of a semicrystalline material was recovered. Several recrystallizations from MeOH–water gave the analytical sample, m.p. 97·5°. (Found: C, 77·08; H, 8·96. $C_{23}H_{32}O_3$ requires: C, 77·49; H, 9·05%); λ_{max} 223, 233 sh., 297 and 340 sh. mμ (ε 15,100, 11,500, 16,000 and 3900); $\nu_{max}^{CHCl_3}$ 1629 cm^{-1}.

Fraction III (LL-Z1272γ). The III fraction was concentrated to a small volume from which crude crystals were obtained. Five grams of crystalline III were obtained by recrystallization from acetone–hexane, m.p. 161–163°. The analytical sample had m.p. 172–173°, $[\alpha]_D^{25} = -31°$ (c = 0·99 MeOH). (Found: C, 68·20; H, 7·21; Cl, 9·30. $C_{23}H_{29}O_4Cl$ requires: C, 68·22; H, 7·22; Cl, 8·74%); λ_{max} 230, 293 and 347 mμ (ε 35,700, 11,370 and 10,150); $\nu_{max}^{CHCl_3}$ 1706 and 1629 cm^{-1}.

Fraction IV (LL-Z1272δ). Concentration of the IV fraction to a small volume provided a crystalline mixture which consisted mostly of IV with some III. Fractional crystallization from acetone–hexane gave 5·3 g of IV, m.p. 129·5–130·5°. $[\alpha]_D^{25} = +6°$ (c = 1·0 MeOH). (Found: C, 67·93; H, 7·81; Cl, 9·08. $C_{23}H_{31}O_4Cl$ requires: C, 67·89; H, 7·68; Cl, 8·71%); λ_{max} 231, 293 and 346 mμ (ε 23,000, 12,000 and 9150); $\nu_{max}^{CHCl_3}$ 1706 and 1629 cm^{-1}.

Fractions V *and* VI (LL-Z1272ε *and* ζ). The V and VI fraction, when concentrated to dryness gave an oil which failed to crystallize. This oil was rechromatographed on a Celite partition column using the system MeOH–heptane, and the effluent was monitored at 292 mμ. The V fraction was concentrated to dryness providing material which crystallized from AcOEt–hexane to give 8·3 g of V, m.p. 171·5–172·5°. $[\alpha]_D^{25} = +6°$ (c = 0·93 MeOH). (Found: C, 73·15; H, 8·62. $C_{23}H_{32}O_4$ requires: C, 74·16; H, 8·66%); λ_{max} 223, 233 sh., 295 and 340 sh. mμ (ε 15,600, 11,500, 16,500 and 3700). $\nu_{max}^{CHCl_3}$ 1709 and 1629 cm^{-1}. Mass spectrum exhibited a molecular ion at *m/e* of 372·228 in accord with the molecular formulation of $C_{23}H_{32}O_4$.

Concentration of the VI fraction gave a semicrystalline solid which yielded 4·0 g of VI from acetone–hexane, m.p. 156·5–157°, $[\alpha]_D^{25} = -15°$ (c = 1·0 MeOH). (Found: C, 65·01; H, 7·03; Cl, 8·02. $C_{25}H_{31}O_6Cl$ requires: C, 64·86; H, 6·75; Cl, 7·66%); λ_{max} 239, 293 and 347 mμ (ε 39,800, 11,700, 9600); $\nu_{max}^{CHCl_3}$ 1740, 1715 and 1629 cm^{-1}.

Formation of the chromane derivatives VII *and* VIII *from* V

To 8 ml of concentrated H_2SO_4 was added 200 mg of V and the soln allowed to stand at room temp

for 30 min. This was then poured onto cracked ice and the mixture extracted with CH_2Cl_2. The extract was washed with water, dried, and concentrated to a residue, which was then chromatographed on silica gel. Elution with 2% AcOEt in benzene gave 50 mg of chromane VII as a gum; λ_{max} 221 sh and 297 mμ (ε 10,000 and 13,000); ν_{max}^{KBr} 1705, 1630 and 1580 cm^{-1}. Subsequent elution with 20% AcOEt in benzene gave 67 mg of chromane VIII as white crystals, m.p. 185–190°. Both compounds showed single spots on TLC (silica gel, 10% AcOEt in benzene, detected by UV), having R_f's of 0·45 (VIII) and 0·85 (VII).

Recrystallization of VIII from benzene–hexane gave the analytical sample, m.p. 192–194°, $[\alpha]_D^{25} = 0°$ (c = 0·475 $CHCl_3$). (Found: C, 73·86; H, 8·47. $C_{23}H_{32}O_4$ requires: C, 74·16; H, 8·66%); λ_{max} 224, 233, 286 and 315 sh. mμ (ε 13,000, 13,400, 13,400 and 6340); ν_{max}^{KBr} 1710, 1667, 1640, and 1587 cm^{-1}.

Ozonolysis of I (LL-Z1272α). A 250 mg sample of I in 50 ml of MeOH was ozonized at −70° for 30 min. The dark-blue solution was then flushed with N_2 and treated with Me_2S at −70°. The temp was raised and held at −10° for 1 hr, then 0° for 1 hr, and finally, room temp for 1 hr.[16] The solution was then directly distilled at 66° into a 2,4-dinitrophenylhydrazine trap to give 90 mg of crude acetone-2,4-DNP (59%—1 mole) m.p. 113–120°. Recrystallization from 95% EtOH gave the pure derivative, m.p. 120–122°, which was identical with an authentic sample (IR, m.p., mixed m.p. and TLC).

The oil remaining in the distillation flask was slurried with water forming a semicrystalline ppt. This was filtered into 2,4-DNP reagent to give 360 mg of crude levulinic aldehyde di-2,4-DNP (57%—2 moles), m.p. 222–227°. Recrystallization from nitrobenzene gave the pure derivative, m.p. 234–238° which was identical with an authentic specimen (IR, m.p., mixed m.p. and TLC).

The water-insoluble crystalline residue was recrystallized from MeOH to give 38 mg of the dihydrobenzofuran X (identical to the dihydrobenzofuran obtained from the ozonolysis of III as shown by IR, m.p., mixed m.p. and TLC) (see below).

Ozonolysis of III (LL-Z1272γ). A 1·0 g sample of III in 150 ml of MeOH was ozonized at −70° for 1 hr. The dark-blue soln was then flushed with N_2 and treated with 5 ml of Me_2S at −70°. The temp was raised and held at −10° for 1 hr, then 0° for 1 hr, and finally room temp for 1 hr. The soln was concentrated to a semicrystalline residue which was dissolved in CH_2Cl_2 and extracted with 10% NaOH aq.

The CH_2Cl_2 phase was washed with water, dried with Na_2SO_4, and concentrated to give 417 mg of an oil. This was chromatographed on a Celite partition column employing the system heptane–MeOH to give 258 mg of crude XIII. Evaporative distillation at 125°/10^{-2} mm gave 220 mg of purified XIII which gave a positive iodoform test (iodoform identified by m.p. and mixed m.p. with an authentic sample). The mass spectrum exhibited a molecular ion at m/e 208 in accord with the molecular formulation $C_{13}H_{20}O_2$. λ_{max} 223 mμ (ε 12,000); ν_{max}^{film} 1720, 1690 and 1634 cm^{-1}.

The basic aqueous extract was acidified with 6N HCl and the soln extracted with ether. The ethereal extract was washed with water, dried with Na_2SO_4, and concentrated to a semicrystalline residue. Trituration of this residue with MeOH gave a crystalline solid, m.p. 163–168°. Recrystallization from MeOH gave the analytical sample of X (66 mg), m.p. 170–172°. (Found: C, 54·81; H, 4·80; Cl, 14·68. $C_{11}H_{11}O_4Cl$ requires: C, 54·44; H, 4·57; Cl, 14·61%); λ_{max} 266, 293 and 335 sh mμ (ε 16,300, 13,100 and 4000); ν_{max}^{KBr} 1640 cm^{-1}; NMR: 138 Hz (s, 3H), 180 (m, 2H), 215 (s, 3H), 355 (m, 1H), 610 (s, 1H), 747 (s, 1H).

The structure of X was further substantiated by conversion to the corresponding benzofuran derivative. A sample of the dihydrobenzofuran X (100 mg) was slurried in 2 ml of 85% H_3PO_4 and warmed on the steam bath for 10 min. The reaction mixture was poured over crushed ice and then extracted with benzene. The benzene extract was washed with water, dried with Na_2SO_4 and concentrated to a residue which was then chromatographed on 10 g of silica gel. The benzofuran derivative was eluted with benzene and gave after recrystallization from dilute EtOH, 4 mg of crystalline benzofuran, m.p. 112–118°; λ_{max} 238, 250, 284 and 350 mμ (ε 12,400, 11,200, 3150 and 1470); NMR: 163 Hz (s, 3H), 420, 455 (AB, J_{AB} = 2·5 Hz), 618 (s, 1H), and 782 (s, 1H).

Catalytic reduction of III *and* IV (LL-Z1272γ, δ) *and comparison of their reduction products*

A soln of 200 mg of III in 50 ml of 9:1 cyclohexane–EtOH, was stirred overnight with 50 mg of 10% $Pd/CaCO_3$ under an atmosphere of H_2, during which time approximately 2 moles of H_2 was adsorbed. After filtration to remove the catalyst and evaporation of the solvent, the crude reduction product was subjected to partition chromatography on an 80 g Celite column using a heptane–MeOH (sat'd. with $AgNO_3$) solvent system. The fractions containing the main reduction product, detected by UV at 290 mμ, were combined and concentrated to give 130 mg of clear, nearly colorless glass. A 200 mg sample of IV when treated similarly, absorbed about 1 mole of H_2 and gave, after partition chromatography, 93 mg of a clear, pale yellow glass.

Further purification of the reduction products was achieved by silica gel chromatography with the major reduction product, in each case, eluted as a colorless band with 1:1 CCl_4–$CHCl_3$. Upon removal of the solvent, a clear, colorless viscous oil was obtained from both samples which were indistinguishable by TLC (Eastman Chromogram K301R, 3:1 $CHCl_3$–hexane to develop, UV and I_2 to detect) each showing a single spot with R_f 0·40. Comparison of their IR, UV and NMR spectra also showed them to be identical.

Formation of the base product XVI *from* VI (LL-Z1272ζ)

To a warm soln of 2·0 g of VI in 80 ml of MeOH, was added 80 ml of 0·1N NaOH. The soln was warmed on the steam bath for 1 hr whereupon it was cooled and acidified (congo red) with 5N HCl. Extraction with 2 × 200 ml of ether followed by drying with Na_2SO_4 and evaporation gave a gummy residue. Crystallization from warm MeOH gave 1·44 g of impure XVI. Chromatography on a Celite partition column (1 Kg) using the system MeOH–heptane provided 1·0 g of XVI from MeOH. The analytical sample had m.p. 125–130° and 160–163°. (Found: C, 68·37; H, 6·79; Cl, 8·87. $C_{23}H_{27}O_4Cl$ requires: C, 68·56; H, 6·75; Cl, 8·80%); λ_{max} 234, 292 and 347 mμ (ε 45,300, 12,900, 9500); ν_{max}^{KBr} 1675 and 1637 cm^{-1}.

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