Photoisomerization of Diester 3b. Freshly recrystallized *trans,-cis,cis,trans* diester **3b** (0.50 g) was dissolved in cold (0°) ethyl acetate (60 ml) and the solution was flushed with nitrogen for 30 min. The solution was then irradiated (Srinivasan-Griffin chamber reactor, 3500-Å lamps) for 20 min at 0 to −10°. The solution was concentrated under reduced pressure to a volume of *ca.* 8 ml and chilled. Filtration gave the all-*trans* ester **6** (0.48 g, 95%), mp 210–213°. Recrystallization gave the pure all-*trans* dimethyl 2,4,6,8-decatetraene-1,10-dioate as tiny yellow prisms: mp 212–213°; ir (KBr) 1700 (C=O), 1617 (C=C), and 1302 (C—O) cm^{-1}. A mixture melting point with an authentic sample[8] was undepressed.

Thermal Isomerization of Diester 3b to Bicyclic Ester 5b. A solution of freshly recrystallized diester **3b** (0.50 g) in dry benzene (50 ml) was flushed with nitrogen for 1 hr and was then refluxed under nitrogen for 8 hr, all in a darkened room. Evaporation of the solvent under reduced pressure and trituration of the residue with ether–pentane gave the crude product as a very pale yellow semisolid. Recrystallization from ether–pentane at −20° gave **5b**, *trans*-7,8-dicarbomethoxybicyclo[4.2.0]octa-2,4-diene, as colorless prisms (0.31 g, 62%): mp 34–35°; ir (CCl_4) 1726 (C=O) and 1170–1200 (C—O) cm^{-1}; uv (CH_3OH) max 270 nm (ε 3000); nmr ($CDCl_3$) τ 4.0–4.7 (4 H, multiplets, vinyl hydrogens), 6.05–6.24 (2 H, multiplets, H-7 and H-8) 6.28 and 6.31 (3 H each, singlets, OCH_3); mass spectrum parent ion at *m/e* 222. Evaporation of the mother liquors left an oily residue whose spectral properties suggested it contained dimers of **5b**.

Diels–Alder Reaction of Diester 5b with N-Phenylmaleimide. A solution of diester **5b** (0.30 g, 1.5 mmol) and N-phenylmaleimide (0.25 g, 1.5 mmol) in tetrahydrofuran (5 ml) was allowed to stand in the dark at room temperature for 10 hr. The solution was treated with a few drops of hexane, chilled, and filtered. The white solid thus obtained (0.44 g, 77%) was almost pure adduct. A portion was recrystallized from ethyl acetate to give the analytical sample: mp 185–186° dec; ir (KBr) 1733 (ester C=O) and 1705 (amide C=O) cm^{-1}.

Anal. Calcd for $C_{22}H_{21}NO_6$: C, 66.84; H, 5.32. Found: C, 66.73; H, 5.62.

Hydrolysis of 5b. Isolation of Diacid 5a. A mixture of freshly prepared diester **5b** (0.20 g) and 10% aqueous sodium hydroxide (10 ml) was stirred at room temperature for 36 hr. The solution was extracted once with ether and then cooled and acidified with concentrated hydrochloric acid. The precipitated acid was filtered and washed with cold water and then reprecipitated from basic solution. The off-white solid thus obtained, **5a** (0.11 g, 60%), decomposed at 245–260° without a definite melting point: ir (KBr) 1695 (COOH) and 1245 and 1310 (C–O) cm^{-1}; nmr (0.5 *M* NaOD in D_2O) τ 4.0–4.3 (4 H, m, vinyl hydrogens), 6.2–6.4 (2 H, m, H- and H), and 6.5–6.9 (2 H, m, H-1 and H-6).

Anal. Calcd for $C_{10}H_{10}O_4$: C, 61.87; H, 5.53. Found: C, 61.10; H, 5.14.

Isolation of Ester 10. A mixture of diacid **3a** (5 g), methanol (50 ml), and sulfuric acid (4 drops) was refluxed under nitrogen for 2 hr. Most of the methanol was removed by evaporation under reduced pressure. The residue was taken up in ether, washed once with 5% sodium bicarbonate and with water, and dried. Evaporation of the solvent gave a brown oil which was chromatographed on silica gel (2.5 × 300 nm). Elution with 4:1 and 2:1 hexane–methylene chloride gave several fractions containing colorless oils; the first three of these crystallized on standing. Recrystallization from hexane gave colorless chunky prisms (0.68 g, 11%) of diester **10**: mp 69–70°; ir (KBr) 1708 (C=O) and 1572 (C=C) cm^{-1}; nmr (CCl_4) τ 2.28 (2 H, S, vinyl H), 6.20 (6 H, s, OCH_3), 6.3 (2 H, M, CH), and 7.1–7.4 (4 H, M, $-CH_2-$); uv (EtOH) max 327 nm (ε 9200).

Later fractions contained somewhat impure diester **5b**; in three of five runs under these conditions, only **5b** was obtained.

Acknowledgments. The author wishes to thank the Robert A. Welch Foundation for financial support of this work and the Badsiche Anilin-und-Soda Fabrik Co. for a generous gift of cyclooctatetraene. Thanks are also due to Professor Elliott N. Marvell for helpful discussions.

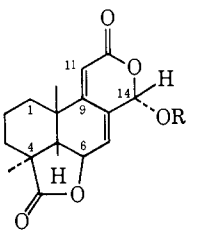
Structure and Chemistry of Antibiotic LL-Z1271α, an Antifungal Carbon-17 Terpene

George A. Ellestad, Ralph H. Evans, Jr., Martin P. Kunstmann, John E. Lancaster, and George O. Morton

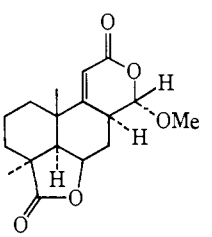
Contribution from Lederle Laboratories, A Division of American Cyanamid Company, Pearl River, New York 10965.
Received February 5, 1970

Abstract: The structure of a novel $C_{17}H_{20}O_5$ terpenoid antifungal agent I, labeled LL-Z1271α, is elucidated. In addition, the chemistry of some interesting base hydrolysis products of this mold metabolite is discussed.

In a continuing search for biologically useful fungal metabolites we had occasion to examine the metabolic products from fermentations of an unidentified *Acrostalagmus* species. From this investigation we isolated a novel C_{17} terpenoid I, labeled LL-Z1271α,[1] which exhibited *in vitro* and *in vivo* antifungal activity against several experimental fungal infections. However, later test results indicated considerable toxic side effects as well. Though the structure was determined primarily by physical methods in conjunction with biogenetic considerations, several intriguing base-catalyzed transformations were encountered giving rise to products which at first tended to obscure rather than aid our structural deductions. But as seen below, the structures of these derivatives, as well as their genesis, are singularly consistent with I.



I, R = Me
II, R = H
III, R = Ac

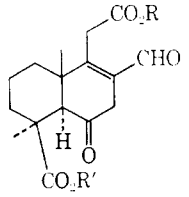


IV

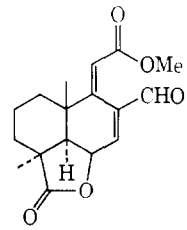
(1) A portion of this work was reported in preliminary form: G. A. Ellestad, R. H. Evans, Jr., and M. P. Kunstmann, *J. Amer. Chem. Soc.*, 91, 2134 (1969).

Mass spectrometry and microanalysis of I established its molecular formula as $C_{17}H_{20}O_5$. The nmr spectrum indicated the presence of two tertiary C-methyl groups, with signals at δ 1.16 and 1.33, and strongly suggested that I was terpenoid in nature. A three-proton singlet at δ 3.70 indicated a methoxy group, and a broadened one-proton triplet at δ 5.07, a two-proton signal at δ 5.76, and a complex one-proton signal at δ 6.53 were evidence of four protons on double bonds and carbon atoms bearing oxygen. The ir spectrum disclosed the presence of a γ-lactone (1775 cm^{-1}) and an additional carbonyl group (1730 cm^{-1}). Because I was unreactive toward sodium borohydride, the latter carbonyl seemed to be due to another lactone or ester function, and the strong uv maximum at 257 nm (ϵ 13,500) suggested that some type of diene or diene lactone or ester function might be responsible for the chromophore. Catalytic reduction with 5% rhodium-on-alumina provided several products, only one of which, the dihydro derivative IV, was isolated in any yield. Absorption at 1775 and 1735 cm^{-1} in the ir of IV showed both carbonyl groups to be intact, but the uv maximum at 218 nm (ϵ 9950) was characteristic of an α,β-unsaturated lactone or ester grouping and pointed to the presence of a diene ester or lactone in the original chromophore.

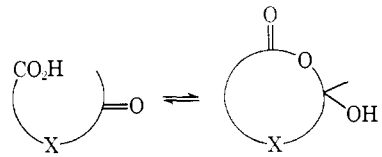
The pseudoester nature of the 1730-cm^{-1} carbonyl group was indicated by the formation of the lactol II, $C_{16}H_{18}O_5$, on mild basic hydrolysis of I with 0.05 *N* sodium hydroxide in 50% methanol. Its ir spectrum (KBr) showed bands at 1780 and 1680 cm^{-1} though the latter absorption was seen at 1720 cm^{-1} when the spectrum was taken in chloroform. A minor product V, $C_{17}H_{22}O_6$, was isolated from this reaction and will be discussed later. The lactol II was also isolated from a few fermentations in small yield and is called LL-Z1271γ. It was characterized by acetylation with acetic anhydride–pyridine to give the lactol acetate III, $C_{18}H_{20}O_6$, with ir absorption at 1775 (sh), 1760, and 1725 cm^{-1}. Treatment of II with ethereal diazomethane, with a little methanol added to aid solution, yielded I and the ester–aldehyde VIII, $C_{17}H_{20}O_5$, as shown by the C-14 aldehyde signal at δ 9.55 in the nmr spectrum of VIII and the appearance of the aldehyde carbonyl band at 1690 cm^{-1} in its ir spectrum. The formation of the pseudoester I and the ester–aldehyde VIII by reaction of II with diazomethane is typical of compounds capable of equilibria of the type IXa–IXb.[2]



V, R = H; R' = Me
VI, R = R' = Me
VII, R = R' = H



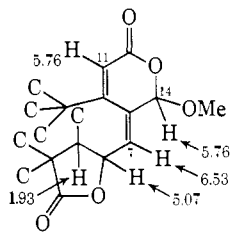
VIII



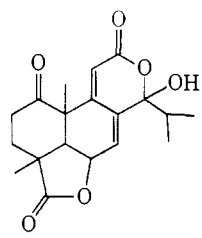
IXa IXb

(2) R. B. Woodward and H. Baer, *J. Amer. Chem Soc.*, 70, 1161 (1948), and references therein.

Spin-decoupling experiments in deuteriobenzene (in this solvent the C-11 and C-14 proton signals were resolved), in conjunction with the chemical shifts and magnitude of the coupling constants of the four low-field protons, enabled us to make assignments of these signals and to define the relationship between the γ-lactone, diene, and pseudoester grouping as depicted in the partial structure **X**. The uv maximum of 259 nm in **XI**[3] provided strong support for the chromophore.[4]



X

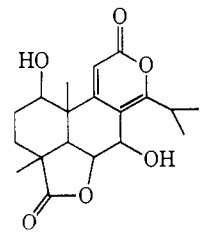


XI

$J_{5,6} = 5.3$ Hz
$J_{6,7} = 4.7$ Hz
$J_{6,14} = 1.0$ Hz
$J_{7,14} = 2.0$ Hz
$J_{7,11} = 1.8$ Hz

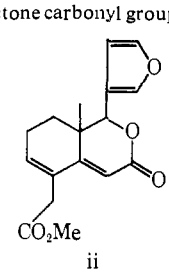
The presence of two carbocyclic rings is dictated by the molecular formula, the functional groups noted, and the lack of additional unsaturation in I. The previously mentioned terpenoid nature of the metabolite, together with the nmr and chemical evidence, led us to consider structures I, XII, XIII, XIV, and XV for LL-Z1271α though on biogenetic grounds the A/B *cis*-fused possibilities seemed unlikely. Any *trans* diaxial H-5,6 structures can be ruled out since, in general, $J_{5,6}$ values for this orientation in known diterpene C-4,6 γ-lactones are found in the range of 11.0–13.0 Hz whereas *cis* protons have *J*'s of 4.6–8.0 Hz.[5]

(3) Compound XI is formed from nagilactone A (i) by oxidation with chromic acid in pyridine. Nagilactone A is obtained from the seeds and leaves of *Podocarpus nagi*, Zoli and Moritzi: Y. Hayashi, S. Takahashi, H. Ona, and T. Saken, *Tetrahedron Lett.*, 2071 (1968).



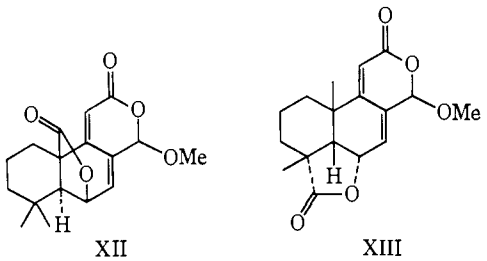
i

(4) The uv maxima in these compounds are considerably lower than that found for the corresponding chromophore in ii (λ_{max} 272 nm (ϵ 15,-200)); J. D. Connolly, R. McCrindle, K. H. Overton, and W. D. C. Warnock, *ibid.*, 2937 (1965). This difference must be due to the oxygen atom in the ϵ position to the lactone carbonyl group and seems analogous to the



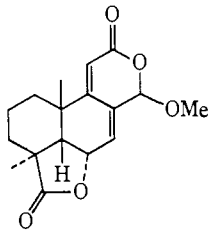
ii

well-known hypsochromic shift of α,β-unsaturated γ-hydroxy ketones; T. A. Geissman and G. A. Ellestad, *J. Org. Chem.*, 27, 1855 (1962); T. G. Halsall, W. J. Rodewald, and D. Willis, *J. Chem. Soc.*, 2798 (1959); R. J. Conca and W. Bergmann, *J. Org. Chem.*, 18, 1104 (1953).

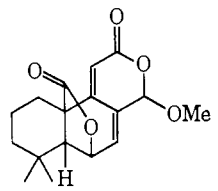


XII

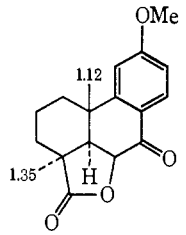
XIII



XIV

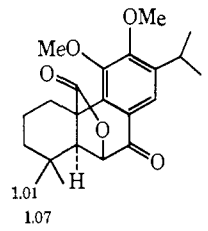


XV



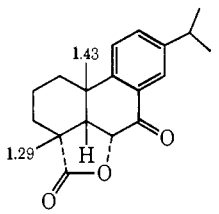
XVI

$J_{5,6}$ = 5.5 Hz



XVII

$J_{5,6}$ = <1 Hz



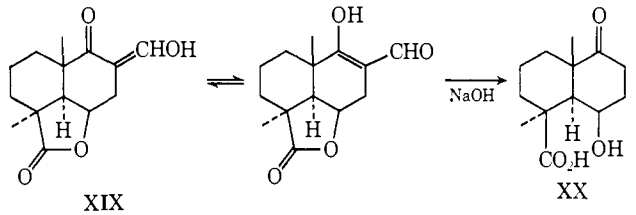
XVIII

$J_{5,6}$ = 8.0 Hz

Structure I was chosen primarily on the basis of the H-5,6 coupling constant (5.3 Hz) and chemical shifts of the C-4 (δ 1.33) and C-10 (δ 1.16) methyl groups in the nmr spectrum of LL-Z1271α. These values are in good agreement with the corresponding assignments in the podocarpic acid derivative **XVI** ($J_{5,6}$ = 5.5 Hz and δ 1.35 and 1.12).[5a,c] Also, the pseudoequatorial assignment of the C-6 hydrogen in I is consistent with the $J_{6,7}$ of 4.7 Hz.[6,7] The $J_{5,6}$ value of less than 1 Hz in the nmr spectrum of the carnosol-related lactone **XVII**[5a] suggests a nearly 90° dihedral angle between the C-5 and C-6 protons in that molecule whereas the corresponding J of 8.0 Hz in the dehydroabietic acid derivative **XVIII**[5b] indicates an H-5,6 dihedral angle of near zero degrees. Models of **XVIII** reveal a more complex conformational situation than in the A/B *trans* structures but clearly show the C-10 methyl group is removed from the anisotropic influence of the γ-lactone function. Although there are no literature analogies for the structure and stereochemistry depicted in **XIV** and **XV**, Dreiding models serve to show conclusively that the magnetic environment of the C-methyl groups in these compounds would be considerably different than in **XVI** and I.[8]

The axial nature of the C-14 proton in I is suggested by the allylic $J_{7,14}$ value of 2.0 Hz[6,7] in the nmr spectrum of I. Consistent with this assignment is the vicinal $J_{8,14}$ splitting constant of 9.0 Hz in the spectrum of dihydro I (IV) which points to a *trans* diaxial relationship of the C-8 and C-14 protons in that molecule.[9] On the basis that reduction of the Δ^7 double bond in I would occur from the side opposite the substituents at C-4, C-6, and C-10,[10] the axial C-14 hydrogen must be *cis* to these functionalities in IV and therefore in I. A circular dichroism curve of IV shows a negative Cotton effect at 255 nm ([θ] − 2.2 × 10⁴) for the enelactone chromophore which indicates C-14 to have the absolute configuration as depicted.[9]

Circular dichroism studies on **XX** enabled us to determine the absolute configuration of the remaining asymmetric centers. Ozonolysis of IV gave **XIX** which was deformylated with base to give, in small yield, the hydroxy keto-acid **XX**. The negative Cotton effect ([θ] − 2.52 × 10³) at 291–293 nm in the curve of **XX** requires, from the octant rule,[11] a β C-10 methyl group which, at the same time, defines the absolute stereochemistry at C-4, C-5, and C-6 on the basis of the previously established relative stereochemistry of these centers. The C-14 assignment which is likewise determined from this result is in agreement with that obtained above by independent means.



XIX XX

The chemical nature of V was uncertain until after the structure of I was clear. Its ir spectrum (KBr) showed carbonyl absorption at 1725 (br) and 1660 cm⁻¹ and its uv spectrum had a maximum at 237 nm (ε 7800) consistent with the acid–aldehyde–ester structure. However, no aldehyde or carboxyl proton was observed in the nmr spectrum indicative that the lactol form predominates in deuteriochloroform. The melting point of ~105° for V with gas evolution is readily explained by loss of CO_2 from the β,γ-unsaturated acid–aldehyde

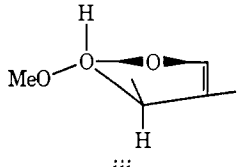
(5) (a) E. Wenkert, A. Fuchs, and J. D. McChesney, *J. Org. Chem.*, 30, 2931 (1965); (b) E. Wenkert and B. L. Mylari, *ibid.*, 30, 4387 (1965); (c) A. E. Lickei, A. C. Rieke, and D. M. S. Wheeler, *ibid.*, 32, 1647 (1967); (d) J. R. Hanson, *Tetrahedron*, 22, 1701 (1966); (e) K. Mori and M. Matusi, *Tetrahedron Lett.*, 1633 (1966); (f) R. Henderson, R. McCrindle, K. H. Overton, and A. Melera, *ibid.*, 3969 (1964); (g) A. Tahara and K. Hirao, *Chem. Pharm. Bull. (Tokyo)*, 15, 1145 (1967); (h) S. Ito, M. Kodama, M. Sunagawa, T. Takahashi, H. Imamura, and O. Hond, *Tetrahedron Lett.*, 2065 (1968).

(6) E. W. Garbisch, *J. Amer. Chem. Soc.*, 86, 5561 (1964).

(7) S. Sternhell, *Rev. Pure Appl. Chem.*, 14, 15 (1964).

(8) For a wealth of data on chemical shifts of C-methyl groups in diterpenes, see C. H. Brieskorn, A. Fuchs, J. B-son Bredenberg, J. D. McChesney, and E. Wenkert, *J. Org. Chem.*, 29, 2293 (1964); E. Wenkert, A. Afonso, P. Beak, R. W. J. Carney, P. W. Jeffs, and J. D. McChesney, *ibid.*, 30, 713 (1965); C. R. Narayanan and N. K. Venkatasubramanian, *Tetrahedron Lett.*, 3639 (1965).

(9) This is on the basis that ring C in IV exists as in projection iii;



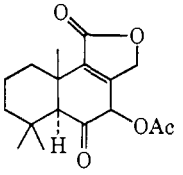
iii

see G. Snatzke, *Angew. Chem., Int. Ed. Engl.*, 7, 14 (1968), which is in turn based on X-ray studies of δ-lactones which show the –C–CO–O– grouping to be planar; K. K. Cheung, K. H. Overton, and G. A. Sim, *Chem. Commun.*, 634 (1965), and references therein. Models of IV fulfilling this requirement suggest an H-8,14 dihedral angle of ~165°.

(10) See R. H. Bible, Jr., and R. R. Burtner, *J. Org. Chem.*, 26, 1174 (1961), who have shown that hydrogenation of podocarpic acid occurs from the α side of the molecule.

(11) W. Moffitt, R. B. Woodward, A. Moscowitz, W. Klyne, and C. Djerassi, *J. Amer. Chem. Soc.*, 83, 4013 (1961). For a close analogy of XX see L. Mangoni and M. Adinolff, *Gass. Chim. Ital.*, 98, 122 (1968).

grouping which provides for facile decarboxylation. In fact, the molecular ion in the mass spectrum of V is not observed; the highest *m/e* is found at 278 (M − 44). As V was difficult to characterize, esterification with diazomethane gave a nicely crystalline diester aldehyde which was clearly VI by ir (1740, 1735, 1710, and 1670 cm^{-1}), nmr, and mass spectrometry (*m/e* 336). Its nmr spectrum, with the exception of the CHO signal at δ 10.00, exhibits no low-field proton signals. The chemical shift of the C-5 proton at δ 2.57 is almost identical with that published for the C-5 hydrogen in **XXI**[12] indicating a *trans*-ring juncture in VI. Inversion at C-5 to a *cis* juncture makes this proton equatorial and coplanar to the adjacent carbonyl group at C-6 and hence, its signal would be expected to resonate at ~30 Hz lower field as seen in **XXIII** (discussed below).

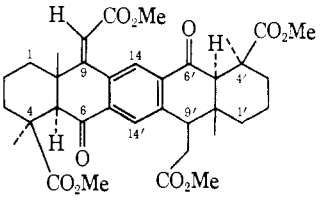


XXI

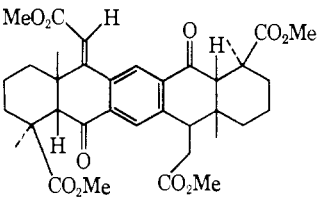
Signals of two overlapping sets of AB systems can be discerned in the nmr spectrum of VI at δ 3.16 (J = 22.5 Hz) and at δ 3.70 (J = 15.0 Hz)[13] to which we have assigned the C-7 and C-11 methylene groups, respectively, on the basis of the following argument. It is well established that in rigid systems, π-bond contribution to the geminal coupling constants of adjacent methylene hydrogens is a maximum when the HH axis of the CH_2 group is parallel to the p orbital of the α sp^2 carbon atom.[14] Models of VI show that the plane of the Δ^8 double bond bisects the angle between the CH bond of the C-7 methylene group and hence contributes a maximum negative ΔJ. The corresponding relationship with the C-6 carbonyl group is not quite so favorable although the likely ring distortion caused by the C-4 carbomethoxy and C-10 methyl interaction would seem to improve this angle for a more negative ΔJ also. In any event, $J_{7,7}$ would be expected to be quite small. On the other hand, the acyclic C-11 methylene group would be expected to have a larger J because it seems unlikely that there would be a large enough population of extreme conformers for the adjacent π system to have a significant effect.

Formation of V can be explained by methanolysis of both γ-lactone and pseudoester groupings with the opening of the γ-lactone ring, the key which sets in motion the necessary prototropic shifts leading to V. Subsequent saponification of the postulated C-12 carbomethoxy intermediate seems reasonable because of its sterically exposed nature as opposed to the notoriously resistant C-4 axial carbomethoxy group.[15] The position of the double bond is of interest as one might expect the Δ^7 isomer to be more stable. Apparently the lower ring strain in the Δ^8 system more than offsets the electronic stabilization in the fully conjugated structure.

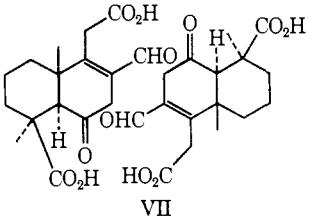
Attempts to obtain larger quantities of VI for additional characterization by conducting the hydrolysis of I in 10% aqueous potassium hydroxide at room temperature followed by esterification with diazomethane led not to VI but instead to a crystalline mixture of stereoisomers with the formula $C_{36}H_{44}O_{10}$ as shown by mass spectral and elemental analyses. Thin-layer chromatography (tlc) showed the presence of two main components and by the use of preparative tlc these were isolated in fair amounts. The front running spot corresponded to the *trans–trans* structure **XXII** and was followed by a second spot which was composed of ~four *cis–trans* isomers which we were unable to resolve in a variety of solvent systems. A trace amount of a third material (the most polar) was also observed though we did not isolate it from the room-temperature



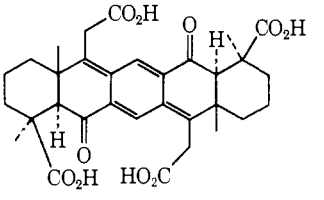
XXII



XXIII



VII



XXIV

reaction. When the reaction was carried out at ~90°, however, this latter substance seemed to be the dominant product and we have assigned it the *cis–cis* structure **XXIII**. The *trans-trans* isomer **XXII** was unstable to sodium methoxide in refluxing methanol from which significant amounts (by tlc) of the *cis–trans* mixture and the *cis–cis* isomer were obtained although the actual isolation of **XXIII** was difficult. The formation of **XXIII** undoubtedly arises due to the relief of the *cis* 1–3 diaxial interactions between the C-4, C-10 and C-4′, C-10′ substituents in **XXII**.

These structural and stereochemical assignments were arrived at primarily on the basis of the nmr spectra of **XXII** and **XXIII**, and on consideration of the structure and possible reactivity of VII which we presumed to be involved. In the *trans–trans* isomer **XXII**, the chemical shifts of the two nonequivalent aromatic protons at δ 7.93 (H-14′) and 8.20 (H-14) are consistent with their strongly deshielded environments due to the *peri* C-6 and C-6′ carbonyl groups and the additional shielding influence on H-14 by the $\Delta^{9(11)}$ double bond. A one-

(12) C. J. W. Brooks and G. H. Draffan, *Tetrahedron*, **25**, 2887 (1969).

(13) Geminal coupling constants are usually negative in sign and thus 22.5 Hz is smaller than 15.0 Hz. For a review of geminal coupling constants see, R. C. Cookson, T. A. Crabb, J. J. Frankel, and J. Hudec, *Tetrahedron, Suppl.*, 355 (1966).

(14) M. Barfield and D. M. Grant, *J. Amer. Chem. Soc.*, **85**, 1899 (1963).

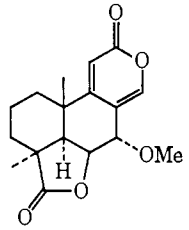
(15) C. L. Graham and F. J. McQuillin, *J. Chem. Soc.*, 4634 (1963); F. E. King, D. H. Godson, and T. J. King, *ibid*, 1117 (1955); W. P. Campell and D. Todd, *J. Amer. Chem. Soc.*, **64**, 928 (1942).

proton singlet at δ 5.97 is assigned to the C-11 proton. Four methoxy signals are seen at δ 3.84 and 3.70 while the C-4 and C-4′ methyls are found at δ 1.53 and 1.57. The C-10′ methyl signal is observed at δ 1.07 and the C-10 methyl, additionally shielded by the $\Delta^{9(11)}$ double bond, at δ 0.90. The H-5′ signal at δ 2.67 is a broadened singlet and is slightly coupled to the benzylic 9′ hydrogen (four σ bonds)[16] at δ 3.55 as demonstrated by spin-decoupling experiments. Irradiation at δ 3.55 caused the two-proton doublet at δ 2.84, assigned to the C-11′ geminal protons, to collapse to a broad singlet and considerably sharpened the H-5′ signal. At the same time the H-14′ and H-14 signals were also sharpened, especially the H-14′ signal at δ 7.93. The H-5 signal is a sharp singlet at δ 2.53.

The nmr spectrum of the *cis–cis* isomer (**XXIII**) is significantly changed from that of **XXII** and reflects the stereochemical changes that have taken place. The H-5 signals are shifted downfield to δ 2.97 and 3.15 as they are now coplanar with the adjacent carbonyl groups. On the other hand, the C-4 methyl signals have moved upfield ~25 Hz since they are curled under the two B rings and are no longer in the deshielding cones of the C-6 carbonyls. The H-11 signal is downfield some 14 Hz to δ 6.10 from its chemical shift in **XXII** and isomerization at C-11 seemed the most plausible explanation as this proton is now *syn* to the aromatic ring and is more deshielded. However, the possibility that other changes in shielding effects due only to the H-5 epimerizations cannot be excluded though this seems less likely.

Compound VII must be the first intermediate in the formation of **XXII** which condenses with itself to give **XXIV**, which is followed by aromatization with either H-11 or H-11′ becoming vinylic. The assigned β nature of the acetic acid side chain at C-9 (or C-9′) is consistent with the fact that axial protonation at C-9′ would occur giving an equatorial side chain rather than the reverse situation.

Alkaline methanolysis of I with sodium methoxide in dry methanol gave an α-pyrone **XXV** in 20% yield. Analysis indicated the formula to be $C_{17}H_{20}O_5$, with ir bands at 1770 and 1720 and the characteristic α-pyrone absorption at 1560 cm^{-1}. The uv maximum at 291 nm (ε 5900) was also consistent with this structure which is formed by a Michael addition of methoxide at C-7 and subsequent elimination of the methoxy group at C-14.



XXV

Analysis of the 100-MHz nmr spectrum of **XXV** showed the H-5 and H-7 signals at δ 4.70 and 4.60, respectively, with $J_{6,7}$ = 3.5 Hz, $J_{5,6}$ = 5.5 Hz, and $J_{7,14}$ = 2.0 Hz. The H_{11} and H_{14} signals were observed at δ 6.00 and 7.62, respectively, with $J_{11,14}$ = 1.0 Hz.[17] The magnitude (2.0 Hz) of the $J_{7,14}$ allylic coupling suggests H-7 is pseudoaxial[6,7] which is consistent with methoxide attack from the α side of the molecule as would be expected.

The isolation of LL-Z1271α is notable in that it is, to our knowledge, the first example of a terpene with a C_{16} carbon skeleton. Biogenetically, it could arise from microbiological degradation of a normal diterpene of the labdane type by oxidative cleavage between C-12 and C-13. A reasonable alternative would be the addition of a C_1 unit to C-11 of a sesquiterpene precursor of the drimenin class.[18]

Experimental Section[19]

Isolation of LL-Z1271α (I). The whole mash from a 300-l. fermentation was adjusted to pH 3.0 with hydrochloric acid and extracted with 135 l. of ethyl acetate. Concentration of the extract to 4 l. *in vacuo* followed by drying over sodium sulfate and the addition of hexane caused 20.5 g of essentially pure LL-Z1271α to crystallize. Recrystallization from either acetone–hexane or methylene chloride–hexane gave the analytical sample with mp 214–215°: $[\alpha]^{25}D$ −203° (*c* 0.29, MeOH); ν_{max}^{KBr} 1775 (γ-lactone) and 1730 cm^{-1} (δ-lactone); λ_{max}^{MeOH} 257 nm (ε 13,500); nmr ($CDCl_3$) δ 1.11 and 1.33 (s, CH_3's at C-10 and C-4), 1.93 (d, H-5, $J_{5,6}$ = 5.3 Hz), 3.70 (s, OMe), 5.07 (t, H-6), 5.76 (2-H's, H-11 and -14), and 6.53 (m, H-7); nmr (benzene-d_6), δ 1.08 and 1.16 (s, CH_3's), 3.60 (s, OMe), 4.73 (t, H-6), 5.52 (t, H-14), 5.70 (d, H-11), and 6.43 (m, H-7); mass spectrum *m/e* 304 ($C_{17}H_{20}O_5$).

Anal. Calcd for $C_{17}H_{20}O_5$: C, 67.09; H, 6.62; O, 26.29. Found: C, 66.93; H, 6.93; O, 25.93.

Isolation of LL-Z1271γ (II). The methanol extracts of the mycelium pads from three 30-l. fermentations were combined and concentrated *in vacuo* to an aqueous phase, which was extracted twice with one-half volumes of chloroform. The chloroform layer was separated, dried over sodium sulfate, and concentrated to 30.5 g of an oily residue. Silica gel chromatography (300 g of slurry packed in methylene chloride) by elution with 1 l. of methylene chloride and 5 l. of ether–methylene chloride (1:4) (monitored at 255 nm) gave two bands. The first gave 1.33 g of I while the second gave 265 mg of II (from ether): mp 241–247°; $[\alpha]^{25}D$ −256° (*c* 0.43, MeOH); ν_{max}^{KBr} 1780 (γ-lactone) and 1680 cm^{-1} (δ-lactol, occurs at 1720 in $CHCl_3$); nmr ($CDCl_3$ with 2 drops of DMSO-d_6) δ 1.16 and 1.33 (s, CH_3's at C-10 and C-4), 1.95 (d, H-5, $J_{5,6}$ = 5.0 Hz), 5.07 (t, H-6), 5.77 (d, H-11, $J_{7,11}$ = 2.0 Hz), 6.08 (broadened s, H-14), and 6.46 (m, H-6); λ_{max}^{MeOH} 257 nm (ε 15,400); mass spectrum *m/e* 290 ($C_{16}H_{18}O_5$).

Anal. Calcd for $C_{16}H_{18}O_5$: C, 66.19; H, 6.25. Found: C, 66.46; H, 6.31.

Dihydro LL-Z1271α (IV). Compound I (513 mg) in acetic acid was hydrogenated over 50 mg of 5% rhodium-on-alumina with absorption of 2.05 mol of hydrogen in 3 hr at which time the reaction was stopped. The catalyst was removed by filtration through Celite and the acetic acid removed *in vacuo*. Trituration with ether gave 163 mg of white crystals. Recrystallization from ethyl acetate–hexane provided 89 mg of the analytical sample, mp 205°, with slight bubbling: $[\alpha]^{25}D$ +97.5° (*c* 0.37, MeOH); ν_{max}^{KBr} 1775 (γ-lactone) and 1735 cm^{-1} (δ-lactone); nmr ($CDCl_3$) δ 1.18,

(16) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, San Francisco, Calif., 1966, p 121.

(17) W. H. Pirkle and M. Dines, *J. Heterocycl. Chem.*, 6, 1 (1969).

(18) H. H. Appel, C. J. W. Brooks, and K. H. Overton, *J. Chem. Soc.*, 3322 (1959).

(19) All melting points were determined on a Fisher–Johns melting point block and are uncorrected. Nmr spectra were recorded with a Varian A60D in $CDCl_3$ (unless otherwise stated) with in some cases a small amount of DMSO added; shifts are expressed as δ values (parts per million) from tetramethylsilane as internal standard, and coupling constants (*J*) are expressed in cycles per second (Hz). In nmr descriptions, s = singlet, d = doublet, t = triplet, m = multiplet, dd = double doublet, and q = quartet. Most of the spin-decoupling work was done on a Varian DP60 although compounds XXII, XXIII, and XXV were studied on a Varian HA100. Infrared spectra (KBr disks) were taken on a Perkin–Elmer Model 137 Infracord and ultraviolet spectra on a Cary Model 11 in methanol. We thank W. F. Fulmor and L. Brancone and associates for the spectral and analytical data and Drs. J. Karliner and G. Van Lear for the mass spectra (direct inlet MS9 (AEI)). Circular dichroism curves were obtained on a Cary 60 spectropolarimeter. We also thank A. C. Dornbush and G. S. Redin and associates for the *in vitro* and *in vivo* testing, Dr. P. Shu and associates for the fermentations and initial processing, and Dr. H. Tresner for the identification of the culture.

1.32 (s, CH_3's at C-10 and C-4), 1.77 (d, H-5, $J_{5,6}$ = 6.0 Hz), 3.64 (s, OMe), 4.99 (d, H-14, $J_{8,14}$ = 9.0 Hz), this latter signal is on top of a broad multiplet also at δ 4.99 (m, H-6), 5.85 (d, H-11, $J_{8,11}$ = 2.0 Hz); λ_{max}^{MeOH} 218, nm (ϵ 9950); mass spectrum *m/e* 306 ($C_{20}H_{22}O_6$); CD (*c* 0.0128, MeOH) $[\Theta]_{255}^{25} - 2.20 \times 10^4$, $[\Theta]_{239} \pm 0$, $[\Theta]_{227} + 2.30 \times 10^4$, $[\Theta]_{217} \pm 0$.

Lactol Acetate III from II. Compound II (100 mg) was dissolved in a solution of 2 ml of pyridine and 1 ml of acetic anhydride. At the end of 1 hr, the reaction mixture was diluted with cold hydrochloric acid and the aqueous solution extracted with ether. The ether extracts were pooled, washed with dilute hydrochloric acid, water, dried over sodium sulfate, and concentrated to a small volume. Crystallization was induced by the addition of hexane to give III: mp 205–210°; $[\alpha]^{25}D$ −145° (*c* 0.16, $CHCl_3$); ν_{max}^{KBr} 1775 sh (γ-lactone), 1760 (acetate), and 1725 cm^{-1} (δ-lactone); nmr ($CDCl_3$) δ 1.20 and 1.35 (s, CH_3's at C-10 and C-4), 1.96 (d, H-5, $J_{5,6}$ = 4.5 Hz), 2.25 (s, AcO), 5.05 (t, H-6), 5.85 (d, H-11, $J_{7,11}$ = 2.0 Hz), 6.47 (m, H-7), and 7.02 (t, H-14); λ_{max}^{MeOH} 257 nm (ϵ 14,000); mass spectrum *m/e* 332 ($C_{18}H_{20}O_6$).

Anal. Calcd for $C_{18}H_{20}O_6$: C, 65.05; H, 6.07. Found: C, 64.79; H, 6.04.

Mild Basic Hydrolysis of I. Compound I (500 mg) slurried in 20 ml of methanol was treated with 20 ml of 0.1 *N* sodium hydroxide. Solution was achieved by warming on the steam bath for 15 min. After the solution cooled to room temperature, it was acidified to congo red with 5 *N* hydrochloric acid and diluted with 25% sodium chloride solution. Extraction with ether followed by drying over sodium sulfate and removal of the solvent *in vacuo* provided 460 mg of crystalline residue. This material was slurried with ether and filtered to give 127 mg of II. Recrystallization from ether gave material identical with LL-Z1271γ isolated from the beer.

The mother liquors from the crystallization of II were chromatographed over silica gel and eluted with 10% ether–methylene chloride which first gave unreacted I followed by a small quantity of material of intermediate mobility. Finally, elution with ether gave the ester-aldehyde V. Two crystallizations from benzene–hexane yielded 22 mg melting at 105° with extensive bubbling and softening from 75 to 85°; λ_{max}^{MeOH} 237 nm (ϵ 7800); nmr ($CDCl_3$) δ 1.20 and 1.30 (s, CH_3's at C-10 and C-4), 2.50 (s, H-5), 3.75 (s, OMe), and 5.93 (broad, OH); mass spectrum *m/e* 278. The ion at 278 (322 − 44) is in agreement with the loss of CO_2 from $C_{17}H_{22}O_6$.

Treatment of V with an ethereal solution of diazomethane gave the dimethyl ester VI from ether–hexane: mp 151–153°; $[\alpha]^{25}D$ +108° (*c* 2.0, $CHCl_3$); ν_{max}^{KBr} 1740 and 1735 (ester), 1710 (saturated ketone), and 1670 cm^{-1} (aldehyde); nmr ($CDCl_3$) δ 1.18 and 1.30 (s, CH_3's at C-10 and C-4), 2.57 (s, H-5), 3.16 (q, H-7's, J_{gem} = 22.5 Hz), 3.75 and 3.85 (s, OMe), 3.70 (q, H-11's, J_{gem} = 15.0 Hz), and 10.0 (s, H-14); mass spectrum *m/e* 336 ($C_{18}H_{24}O_6$).

Formation of I and VIII from II. Compound II (80 mg) in ether was treated with an ethereal solution of diazomethane. On evaporation of the solvent, the residue crystallized. Filtration with ether gave 17 mg of VIII: mp 194–196°; $[\alpha]^{25}D$ −211° (*c* 0.77, $CHCl_3$); ν_{max}^{KBr} 1770 (γ-lactone), 1720 (ester), and 1690 cm^{-1} (aldehyde); λ_{max}^{MeOH} 257 nm very broad (ϵ 9100); nmr ($CDCl_3$) δ 1.13 and 1.32 (s, CH_3's at C-10 and C-4), 1.95 (d, H-5, $J_{5,6}$ = 5.0 Hz), 3.54 (s, OMe), 5.16 (t, H-6), 5.90 (d, H-11, $J_{7,11}$ = 1.8 Hz), 6.97 (dd, H-7, $J_{6,7}$ = 4.0 Hz, $J_{7,11}$ = 1.8 Hz), and 9.55 (s, H-14); mass spectrum *m/e* 304 ($C_{17}H_{20}O_5$).

Compound II (554 mg) dissolved in 20 ml of methanol and 48 ml of ether was treated with an ethereal solution of diazomethane. The solvent was allowed to evaporate and the gummy residue (mixture of I and VIII by tlc in $CHCl_3$–ethyl acetate, 1:1) chromatographed over acid-washed silica gel (100 g) and eluted with a gradient between methylene chloride and 10% ether–methylene chloride. This provided no separation of I from VIII. However, fractional crystallization from ether or methylene chloride–hexane gave 85 mg of I as shown by melting point, mixture melting point, and ir. From the mother liquors VIII contaminated with a little I was obtained.

Formation of XX from IV. Dihydro LL-Z1271α (IV) (500 mg) in chloroform was ozonized at −20° for 45 min. The solvent was removed *in vacuo* at room temperature and the residual gum refluxed for 1 hr with water. Extraction with ether and drying over sodium sulfate gave 338 mg of crude XIX. Compound XIX gave a strong dark purple color with 5% aqueous ferric chloride and an R_f 0.6 on silica gel plates in the solvent system chloroform–ethyl acetate (1:1).

Without further purification, XIX was dissolved in 0.1 *N* sodium hydroxide and refluxed for 45 min. Acidification with hydrochloric acid and extraction with ether gave a crude reaction product from which 20 mg of XX was obtained on crystallization from benzene–hexane. Recrystallization from chloroform–hexane gave the analytical sample: mp 183–187°; R_f 0.3 on silica gel plates in methanol–chloroform (3:7); ν_{max}^{KBr} 1710 (ketone) and 1680 cm^{-1} (H-bonded carboxyl); mass spectrum *m/e* 240 ($C_{13}H_{20}O_4$); nmr ($CDCl_3$ + 1 drop of DMSO-d_6) δ 1.27 and 1.32 (s, CH_3's at C-10 and C-4), 4.49 (m, $W_{h/2}$ = 6 Hz, H-6); CD (*c* 0.341, MeOH) $[\Theta]_{334}^{25} \pm 0$, $[\Theta]_{293-291} - 2.52 \times 10^3$, $[\Theta]_{247} \pm 0$, $[\Theta]_{221} + 1.73 \times 10^3$, $[\Theta]_{210} \pm 0$.

Preparation of XXII. LL-Z1271α (2.0 g) was slurried in 150 ml of 10% aqueous potassium hydroxide and the yellow solution which eventually formed was allowed to stand at room temperature for 4 hr. The dark solution was acidified to congo red with concentrated hydrochloric acid, extracted with ether, and the ether extract dried with magnesium sulfate. The dried ether solution was then treated with an ethereal solution of diazomethane which provided, on evaporation of the solvent, a pale yellow gum. Chromatography over silica gel and elution with 2% ether–methylene chloride followed by 5% ether–methylene chloride gave two main fractions. These were further purified on preparative tlc silica gel plates using the solvent system 20% ethyl acetate–benzene. The plates were developed several times before the two main zones were extracted with acetone and concentrated to dryness. The first zone was recrystallized twice from ethyl acetate–hexane to give 140 mg of XXII: mp 237–238°; $[\alpha]^{25}D$ +22.9° (*c* 0.17, MeOH); ν_{max}^{KBr} 1740 (ester) and 1690 cm^{-1} (ketone); λ_{max}^{MeOH} 260, 277, and 336 nm (ϵ 22,900, 18,950, and 2870); nmr ($CDCl_3$) δ 0.90, 1.07, 1.53, and 1.57 (s, angular C-Me's), 2.53, 2.67 (s, H-5 and H-5′), 2.84 (d, H-11's, J = 6 Hz), 3.5 (br t, H-9′, J = 6 Hz), 3.70 (3 MeO's), 3.84 (s, MeO), 5.97 (s, H-11), 7.93 (d, H-14′, J = 1.0 Hz), 8.20 (s, H-14); mass spectrum *m/e* 636 ($C_{36}H_{44}O_{10}$).

Anal. Calcd for $C_{36}H_{44}O_{10}$: C, 67.91; H, 6.97. Found: C, 68.26; H, 7.05.

The second zone was also extracted with acetone and the resulting gum recrystallized twice from ethyl acetate–hexane to give 100 mg of crystals with mp 252–255°: $[\alpha]^{25}D$ +46.2° (*c* 0.18, MeOH); ir identical with that of XXII; λ_{max}^{MeOH} 265, 283, and 342 nm (ϵ 22,000, 18,700 and 2970); nmr was a combination of the spectra of XXII, XXIII (see below), and at least two other *cis–trans* isomers as shown by the C-Me signals; the mass spectrum was essentially the same as that of XXII.

Anal. Calcd for $C_{36}H_{44}O_{10}$: C, 67.91; H, 6.97. Found: C, 67.97; H, 7.04.

A third zone, XXIII (very faint under uv), was not extracted.

Preparation of XXIII. Compound I (190 mg) was treated with 15 ml of 10% aqueous potassium hydroxide and solution obtained by heating on the steam bath for ~15 min. The dark solution was worked up as described above except that the product was purified by column chromatography only. This gave crystals, mp 228–230°, which on recrystallization from methylene chloride–hexane gave XXIII: 13 mg; mp 231–233°; $[\alpha]^{25}D$ +52.6° (*c* 0.08, MeOH); the mass and ir spectra were essentially the same as found for XXII; λ_{max}^{MeOH} 265, 280, and 345 nm (ϵ 28,000, 23,500, and 3820); nmr ($CDCl_3$) δ 0.87, 1.05, 1.09, and 1.18 (s, angular C-Me's), 2.75 (d, H-11's, J ~ 6 Hz), 2.97 and 3.13 (s, H-5 and H-5′), H-9′ signal is observed with the four MeO's between 3.60 and 3.80, 6.10 (s, H-11), 7.86, and 8.05 (s, H-14′ and H-14).

Partition chromatography using the solvent system 50% methyl cellosolve–methanol/heptane gave no further resolution.

Conversion of XXII to XXIII. Compound XXII (40 mg) dissolved in 10 ml of methanol was treated with 25 mg of sodium methoxide and the solution refluxed for 5 hr. After this time tlc indicated that essentially all of XXII was gone and the formation of two, more polar spots corresponding to the second and third zones mentioned above. The pale yellow solution was evaporated to dryness, slurried with methylene chloride, and acidified with 0.1 *N* hydrochloric acid. The organic phase was washed with saturated salt solution, dried (magnesium sulfate), and concentrated to a gum. Repeated preparative tlc (silica gel) in 20% ethyl acetate–benzene provided a reasonable separation of these materials. The zone corresponding to XXIII was extracted with acetone to give 7 mg of a gum whose nmr was essentially identical with that described above for XXIII.

Reaction of I with Sodium Methoxide to Give XXV. LL-Z1271α (500 mg) in 25 ml of dry methanol was treated with 1 equiv of sodium methoxide and the mixture warmed on the steam bath until solution was complete. It was then cooled and acidified to congo red with 5 *N* hydrochloric acid. The solution was diluted with brine and extracted with methylene chloride. The extract was dried with sodium sulfate, and evaporated to dryness to give a pale

yellow gum. Chromatography over silica gel by gradient elution between methylene chloride and 25% ether–methylene chloride gave 62 mg of XXV, mp 183–185°, from benzene–hexane: $[\alpha]^{25}D$ +19.3° (*c* 0.31, MeOH); ν_{max}^{KBr} 1770 (γ-lactone), 1720 (C=O of α-pyrone), 1640, and 1560 cm^{-1} (C=C, α-pyrone);[20] λ_{max}^{MeOH} 291 nm (ε 5900); 100-MHz nmr ($CDCl_3$) δ 1.08, 1.32 (s, CH_3's at C-10 and C-4), 1.82 (d, H-5, $J_{5,6}$ = 5.5 Hz), 3.60 (s, OMe), 4.60 (dd, H-7, $J_{6,7}$ = 3.5 Hz, $J_{7,14}$ = 2.0 Hz), 4.70 (dd, H-6, $J_{5,6}$ = 5.5 Hz, $J_{6,7}$ = 3.5 Hz), 6.00 (d, H-11, $J_{11,14}$ = 1.0 Hz), 7.62 (dd, H-14, $J_{11,14}$ = 1.0 Hz, $J_{7,14}$ = 2.0 Hz); mass spectrum *m/e* 304 ($C_{17}H_{20}O_5$).

Anal. Calcd for $C_{17}H_{20}O_5$: C, 67.09; H, 6.62. Found: C, 67.33; H, 6.47.

(20) K. Nakanishi, "Infrared Absorption Spectroscopy," Holden-Day, San Francisco, Calif., 1962, p 52.

Molecular Architecture of the Cephalosporins. Insights into Biological Activity Based on Structural Investigations[1]

Robert M. Sweet[2] and Lawrence F. Dahl

Contribution from the Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706. *Received February 28, 1970*

Abstract: Crystal-structure analyses by single-crystal X-ray diffraction methods have been carried out on two representative examples of cephalosporin antibiotics and an example of a biologically inactive cephalosporin derivative. These compounds are all analogous to the penicillins in that they contain a substituted β-lactam fused to a sulfur-containing ring. In addition, the penicillin and cephalosporin antibiotics seem to employ the same mode of action when they inhibit the synthesis of bacterial cell walls. These structural studies have revealed significant stereochemical information related to the dependence of the proposed mechanism upon the lability of the β-lactam amide bond and upon the conformation of the antibiotic in the region of the β-lactam ring. Not only have comparisons been made among active antibiotics, but their structures have also been contrasted to a similar but biologically inactive compound. The most striking structural feature is the large pyramidal character of the β-lactam nitrogen atom in the penicillin and two active Δ^3-cephalosporin antibiotics in contrast to the nearly planar lactam nitrogen in the inactive Δ^2-cephalosporin. The ease of base hydrolysis of the lactam amide bond in these antibiotics correlates with biological activity. This lability is rationalized as being due to decreased amide resonance in the antibiotic β-lactam relative to that in free β-lactams and in the biologically inactive Δ^2-cephalosporin. The presence of this decreased electron delocalization is inferred from C–N and C–O bond length differences and from lactam carbonyl stretching frequency variations among these compounds. It is caused in the penicillins by the observed nonplanarity of the lactam nitrogen atom due to ring fusion and in the cephalosporins by this effect plus electron delocalization due to enamine resonance outside the lactam ring. An analysis of the orientation of the carboxyl groups relative to the β-lactam ring in the molecules studied and in the penicillins indicates that, because of the large variation found, the stereochemical requirements placed on this region by the necessity that these molecules be recognized by the proper enzyme may not be very restrictive. The two Δ^3-cephalosporin antibiotics studied were cephaloridine·HCl·H_2O and cephaloglycine. The derivative investigated of the biologically inactive Δ^2-cephalosporin isomer was phenoxymethyl-Δ^2-desacetoxyl cephalosporin. Cephaloridine·HCl·H_2O, $C_{19}H_{17}O_4N_3S_2$·HCl·H_2O, crystallizes in orthorhombic space group $P2_12_12_1$. Each unit cell contains four formula species and has dimensions a = 11.019 ± 0.003, b = 17.398 ± 0.006, and c = 11.006 ± 0.004 Å. Crystals of cephaloglycine, solvated with one molecule each of acetic acid and water for every cephaloglycine molecule contain four $C_{18}H_{19}O_6N_3S \cdot HO_2CCH_3 \cdot H_2O$ species in a monoclinic unit cell of symmetry C2 and dimensions a = 22.081 ± 0.002, b = 10.296 ± 0.001, c = 11.368 ± 0.001 Å, and β = 108.464 ± 0.004°. The unsolvated Δ^2-cephalosporin, $C_{16}H_{16}O_5N_2S$, packs two molecules to a monoclinic unit cell of symmetry $P2_1$ and dimensions a = 12.922 ± 0.006, b = 5.014 ± 0.003, c = 13.712 ± 0.005 Å, and β = 109.867 ± 0.003°.

A considerable amount of work has been done recently to elucidate the structure and synthesis of bacterial cell walls and to propose the mechanism by which the penicillin (Figure 1a)[3] and cephalosporin (Figure 1b)[3] antibiotics inhibit this synthesis.[4–9] Experiments showed that one of the final steps in bacterial cell wall production is the three-dimensional cross-linking of peptidoglycan strands.[4,5] The enzyme peptidoglycan transpeptidase cleaves the C-terminal D-alanine residue from a short peptide chain which terminates with D-ala-D-ala and replaces it with a particular free amino group fastened to an adjacent peptidoglycan strand. Workers first demonstrated that the cell walls of *S. aureus* grown in the presence of penicillin G contained a larger amount of D-alanine[4,5] and had more

(1) (a) Previous paper reporting preliminary results from this work: R. M. Sweet and L. F. Dahl, *Biochem. Biophys. Res. Commun.*, **34**, 14 (1969); (b) presented in part at the Eighth International Congress of the International Union of Crystallography, Buffalo, N. Y., August 8, 1969; see *Acta Crystallogr., A*, **25**, part S3, S201 (1969).

(2) This manuscript is based in part on a dissertation submitted by R. M. Sweet to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the Ph.D. degree, Jan 1970.

(3) The numbering systems used throughout this article are consistent with those shown in Figure 1.

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