Spectral and Chemical Characterization of Fungal Metabolite LL-N313ζ

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The spectral characteristics of LL-N313ζ, including ^{13}C NMR, ^{1}H NMR, ir, uv, and CD studies, are used to elucidate the structure and stereochemistry of this metabolite as I. The behavior of I under a variety of degradative conditions is examined by subjecting the resultant derivatives to intensive spectral investigation. These studies confirm structure I. A suggestion is also made as to the polyketide origin of this the seventh crystalline metabolite obtained from *Sporormia affinis*.

The fungus *Sporormia affinis* yielded crude fermentation extracts which showed pronounced antifungal activity against *Microsporum canis* infections in animals. Isolation work revealed the presence of six crystalline metabolites, respectively named, LL-N313α, -β, -γ, -δ, -ε, and -η.[1] The major metabolite and also the one exhibiting the most potent antifungal activity was LL-N313β. The other components, with the possible exception of LL-N313α, were found in minor quantities. In later fermentations still another metabolite was detected in isolable quantities. This material, designated LL-N313ζ (I), while it is itself inactive as an antimicrobial agent, may be readily converted to products which have marked antifungal activity. The structure of I, based largely on spectral data, has been briefly reported.[2] The purpose of this paper is to elaborate on that communication and to outline some chemical studies on this metabolite.

Compound I was isolated by subjecting filtered fermentation beer of *Sporormia affinis* to batch carbon adsorption followed by acidic aqueous acetone elution of the carbon bed. The concentrated eluate was chromatographed over silica gel to obtain virtually pure I which crystallizes readily from ethyl acetate. The ir of this material shows a strong carbonyl band at 1680 cm^{-1} which shifts to 1703 cm^{-1} upon formation of a monoacetate consistent with the relationship of the hydroxyl and ketone in I. Three *C*-methyl groups are indicated by the ^{1}H NMR curve with two tertiary methyls at δ 1.08 and 1.23 and a secondary methyl at δ 1.02. A diene system is indicated by the AB pair at δ 6.20 and 5.47 (J = 9.5 Hz) and a virtual singlet at δ 5.72 in conjunction with a strong uv maximum at 242 nm (ε 19900). Borohydride reduction of I gives the dihydro II (no carbonyl in the ir) which has essentially an unchanged uv absorption from that of I. Thus the carbonyl is nonconjugated with the olefinic region of the molecule.

Spin-decoupling studies interrelated protons on carbons 1, 2, 3, 4, 6, 7, and 10. In addition the splitting constants suggested the relative stereochemistry at these centers. The appropriate chemical shifts and J values are summarized in Table I. The protons of C_{12} and C_{13} are closed off from the rest of the molecule and consist of an ABXY system with AB and XY geminal pairs. Extensive spin-decoupling experiments were necessary to resolve these relationships with the result that the two C_{12} protons can be assigned to multiplets at δ 2.86 and 2.11 and the C_{13} pair at δ 3.95 and 4.10. The J values in hertz were determined to be as follows: $J_{12\text{-gem}} = 14.6$, $J_{13\text{-gem}} = 11.5$, $J_{12,13} = 11.2$, 9.0, 1.2, and 3.5.

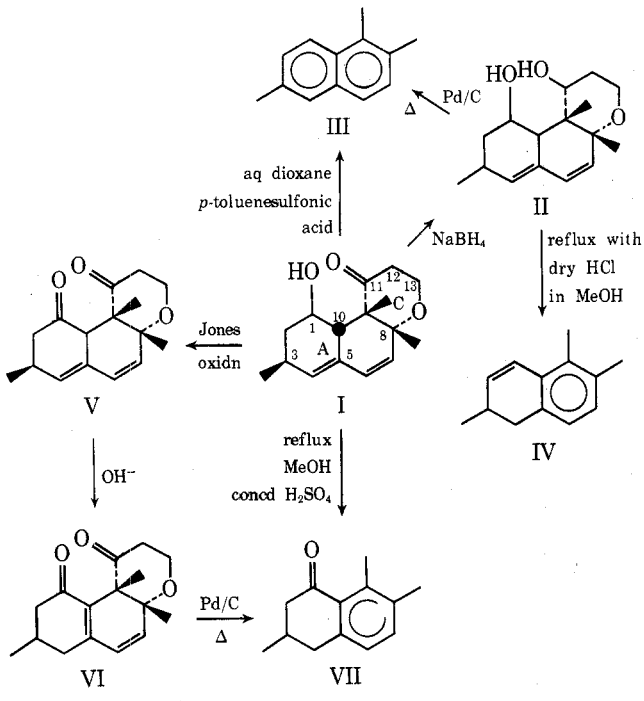
The information so far coupled with the knowledge that carbons 11–13 are removed under a variety of conditions (see below) to give the 1,2,6-trimethyldecalin system in various states of oxidation points strongly to structure I. This structure was confirmed by ^{13}C NMR. The proton noise decoupled 22.6-MHz PFT ^{13}C NMR spectrum of I shows clearly the 16 carbons of LL-N313ζ. Based on the ^{13}C shift data obtained in $CDCl_3$[3] there is only one carbonyl atom in I at 195.5 which is C_{11}. The olefinic resonances at 135.5, 132.1, 130.0, and 128.2 ppm are clearly those associated with the diene. One at least, because of its singlet nature, can be unequivocally assigned to C_5, that is, the C atom with a shift value at 130.0. The carbon with the chemical shift value similar to C_5, that at 132.1, is than logically C_6 because of its comparable situation in the diene system. Of the remaining two carbons of the diene system, C_7 can be assigned the 135.5 signal because of the adjacent alkoxyl grouping at C_8. Singlet multiplicity associated with lines at 78.7 and 57.0 ppm reveals quaternary C atoms, the first of which accounts for C_8 because of its direct link with oxygen and hence the other is due to C_9. The doublets connected with lines at 66.8, 41.7, and 25.3 ppm describe methine C atoms and may be assigned to C_1 which carries a hydroxyl group, C_{10} allylic to a double bond and adjacent to a carbon bearing an oxygen, and C_3 which is simply allylic to a double bond. The methylene carbons with triplet multiplicities at 60.0, 39.3, and 30.6 ppm describe C_{13}, C_{12}, and C_2, respectively, based on proximity to electronegative functionality. Finally the methyl carbons, quartets by off-resonance decoupling with shift values at 21.1, 20.7, and 13.0 ppm, may be tentatively assigned to the methyls at C_8, C_9, and C_3, respectively. There is little doubt that the lowest value is connected with the C_3 methyl but the other two assignments are not so certain.

Table I
^{1}H NMR Data on Protons of Carbons 1, 2, 3, 4, 6, 7, and 10 in I

Proton	Chemical shift	Coupling constants, Hz
H_1	3.95 bm	$J_{1,2e} = 2.0$, $J_{1,2a} = 4.5$
H_{2e}	2.0 m	$J_{gem} = 13.0$, $J_{2e,4} = 1.5$
H_{2a}	1.23 m	$J_{2a,3} = 11.5$
H_3	2.5 bm	$J_{3,CH_3} = 7.0$, $J_{3,2e} = 5.0$
H_4	5.72 bs	$J_{10,4} = 3.0$
H_6	6.20 d	$J_{6,7} = 9.5$
H_7	5.47 d	
H_{10}	3.42 q	$J_{1,10} = 4$

Compound I contains a transoid heteroannular diene system with an allylic oxygen function. According to Beecham[4,5] the chirality of the C=C–C–O determines the sign of the Cotton effect observed with such a system. This is particularly so if the diene system is almost flat, as Dreiding models of I reveal it to be. Burgstahler and Barkhurst, on the other hand, have correlated the chirality of this kind of system with asymmetric perturbation of the double-bond components by allylic axial bonds.[6] Both approaches are attributing the activity primarily to the influence of allylic chirality rather than to the inherent dissymmetry of the chromophore itself. As Beecham points out, it is difficult to see why allylic hydrogens or methyls should outweigh allylic oxygen. Hence, in our communication we

Scheme I
Reactions Resulting in Excision of C Ring from 1 or Related Compounds



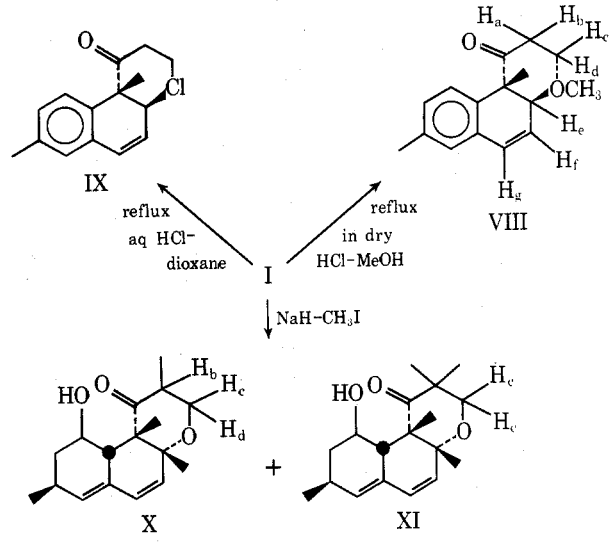
followed Beecham's ideas and since the CD curve of I is a double-humped curve with $\Delta\epsilon_{242.5}$ −21.9 and $\Delta\epsilon_{212.5}$ +21.9, we assigned left-handed chirality to the oxygen-forming helix. An ORD curve on this material shows the following values: ϕ_{267} 0, ϕ_{258} -6.82×10^3, ϕ_{254} 0, ϕ_{229} $+8.29 \times 10^4$, ϕ_{207} 0, ϕ_{203} $+6.82 \times 10^3$, and ϕ_{198} 0.

Examination of the CD and ORD curves shows that they exhibit the symmetry, location, and magnitude of a rotatory couplet, a characteristic form of dispersion which arises when two Cotton effects of equal but opposite strength lie less than a bandwidth apart.[7] While Beecham's ideas concerning allylic oxygen allow us to assign chirality on the basis of CD data, they do not explain the rotatory couplet. Burgstahler's notion of the influence of axial hydrogen or methyl would provide the requisite two chromophores for the couplet. However, both of them in I are skewed in the same direction and hence they would not meet the criterion of oppositeness.

When the Horeau procedure[8] was carried out on I, the recovery of (−)-α-phenylbutyric acid indicated that the configuration of C_1 is *S*. It is also known that the C_1 proton and the C_{10} proton are both cis (J = 4 Hz). The net effect of these facts is strong support for the Beecham interpretation of the CD results. By observing the deshielding shift of δ 0.15 of the C_3 proton in pyridine as opposed to chloroform, we conclude that this proton is 1,3-diaxial relative to the hydroxy group[9] and the secondary methyl is equatorial and hence the complete stereochemistry of I is defined.

As illustrated in Scheme I, when the dihydro derivative II is heated in the presence of palladium on charcoal, the known 1,2,6-trimethylnaphthalene (III) is obtained. The relatively mild procedure of refluxing I in aqueous dioxane in the presence of *p*-toluenesulfonic acid also yields III. When II is refluxed with dry HCl in methanol, IV is obtained. This oil is identified by its spectral data with uv maxima at 223 nm (ε 33500) and 267 (12000). The mass spectrum has a molecular ion peak at *m/e* 172 and metastables at *m/e* 143.3* and 128.4* indicating methyl fragmentations from ions *m/e* 172 and 157, respectively. The ^{1}H NMR spectrum shows a split methyl signal at δ 1.07 and three-proton singlets at δ 2.20 and 2.22 for the virtually

Scheme II
Reactions of I Involving C-Ring Opening or Substitution



equivalent aromatic methyls. The methine proton and benzylic pair are represented by a multiplet centered at δ 2.58. The vinyl protons are an AB pair at δ 5.88 and 6.70 (J = 10 Hz). The signals at δ 5.88 are further split by the methine proton ($J \sim 2$ Hz). The nearly equivalent aromatic protons are given by sharp lines at δ 6.78 and 6.80.

Scheme I illustrates another series of reactions which result in the elimination of the C ring from I. Careful Jones oxidation of I yields V which in the presence of hydroxide ion rearranges to the dienone VI. Despite the fact that VI was obtained chromatographically pure and could be recrystallized to a sharp-melting material, the ^{1}H NMR curves of several preparations always showed minor impurities which hindered interpretation of decoupling studies. Nevertheless we had good evidence for the proposed structure. The uv curve is typical for a conjugated dienone with maximum adsorption at 300 nm (ε 7150); the ir has bands at 1665 cm^{-1} for the conjugated carbonyl and at 1718 cm^{-1} for the pyranone carbonyl. In the ^{1}H NMR curve the tertiary methyls are observed at δ 1.0 and 1.26 while the secondary methyl is a poorly defined doublet at δ 1.10. A multiplet at δ 2.40 integrates for seven protons and the ether methylene pair appears as another multiplet at δ 3.88. The olefinic protons are observed as doublets at δ 5.92 and 6.22 (J = 9.5 Hz).

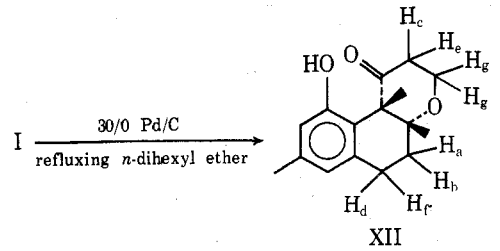
When VI is heated with palladium on charcoal, the tetralone VII is obtained. This oil was also isolated when I was refluxed in dry methanol containing concentrated sulfuric acid. It shows uv maxima at 247 nm (ε 8200) and 298 (2050) and a carbonyl band in the ir at 1675 cm^{-1}. The ^{1}H NMR shows a split methyl at δ 1.08 and an aromatic methyl at δ 2.22 which is where the corresponding methyl signals of IV are located. The other aromatic methyl of VII is shifted to δ 2.40 by virtue of its situation relative to the deshielding cone of the carbonyl. The five protons of the saturated ring are clustered as a broad multiplet around δ 2.40. The two aromatic protons are represented by a narrow AB pair at δ 6.82 and 7.40 (J = 7 Hz).

In Scheme II are illustrated some reactions of I where the C ring is opened or alkylated. In refluxing dry methanolic HCl I yields the oil VIII. The uv of VIII is styrenelike with maxima at 224 nm (ε 25000) and 264 (7000). The ir curve lacks absorption above 3000 cm^{-1} and has a strong carbonyl band at 1712 cm^{-1}. The ^{1}H NMR spectrum shows a split methyl at δ 0.86 (J_{b,CH_3} = 7.5 Hz), a tertiary methyl at δ 1.30, an aromatic methyl at δ 2.32, and a methoxyl singlet at δ 3.25. The H_a and H_b pair are multiplets at δ 2.16

and 2.55 while the ether methylene pair is a multiplet at δ 3.40. Decoupling experiments revealed the coupling constants in hertz for this system: J_{ab} = 17.4, J_{ac} = 5.9, J_{ad} = 6.7, J_{bd} = 7.0, and J_{cd} = 10.0. Proton H_e is a multiplet at δ 2.85 (J_{e,CH_3} = 7.5, J_{ef} = 4.8, and J_{eg} = 1.3 Hz). Vinyl proton H_g appears as four lines at δ 5.90. Upon irradiation of H_e, these four lines coalesce to two lines and the split methyl signal collapses to a singlet. The other vinyl proton is a doublet of doublets at δ 6.28 (J_{gf} = 9.5 and J_{ge} = 1.3 Hz). The aromatic protons are given by one-proton singlets at δ 6.88, 7.02, and 7.04. When I is refluxed in dioxane with aqueous hydrochloric acid, the oil IX is obtained. This relatively unstable β-halo ketone analyzes for $C_{16}H_{19}OCl$ and its uv and ^1H NMR spectra are very similar to those of VIII except of course that the methoxy signal at δ 3.25 in the ^1H NMR spectrum is missing. Compounds VII and IX exhibit good in vitro antimicrobial activity.[11]

We wished to observe the behavior of I under acidic conditions with the hydroxyl group protected as the methyl ether. Treatment of I with sodium hydride and methyl iodide in DMF yields instead of the expected ether the crystalline products X and XI. Mass spectral molecular ions are observed at *m/e* 276 and 290, respectively, and each spectrum exhibits a large metastable peak at *m/e* 143.3* (also observed in spectrum of IV) relating mother and daughter peaks at *m/e* 172 and 157. Both compounds retain the uv chromophore of I and their ^1H NMR spectra have much in common with that of I with diagnostic differences. The spectrum of X has an extra split methyl signal at δ 1.04. H_a is part of a complex signal at δ 2.8 integrating for two protons while H_b and H_c are represented by three lines at δ 3.46 (J = 12 Hz) and four lines at δ 3.96 (J = 8 Hz). Compound XI has two extra methyl signals at δ 0.99 and 1.01. The multiplet at δ 2.8 now integrates for one proton since H_b is missing and the ether methylene pair is represented by two doublets at δ 3.56 and 3.78 (J = 12 Hz).

Under the relatively drastic conditions of Scheme I, the B ring of I or both A and B rings are aromatized along with excision of the C ring. By the milder treatment of Scheme II the A ring is aromatized with loss of oxygen function accompanied by opening of the C ring. In one reaction[12] shown below, the A ring is aromatized without loss of the

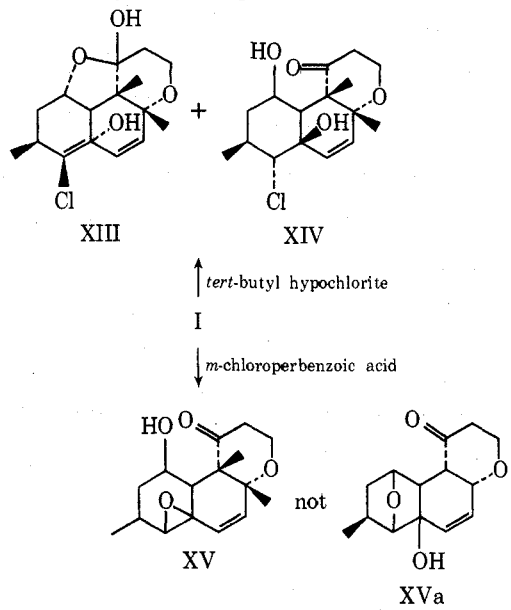


XII

oxygen by removal of hydrogen followed by prototropic shift transfer of the B ring double bond. The mass spectrum of XII shows a molecular ion at *m/e* 260 with peaks of great abundance at *m/e* 188 and 173 and a metastable at *m/e* 159.2*. The ir spectrum shows the carbonyl band at 1695 cm^{-1} while the uv is typical for a phenolic compound with maxima at 215 nm (ε 5400), 278 (1700), and 284 (1800) with a characteristic shift to 293 nm (ε 3000) in alkaline solution. The ^1H NMR spectrum of XII has six readily identifiable signals including the tertiary methyls at δ 1.19 and 1.22, the aromatic methyl at δ 2.21, the exchangeable phenolic proton at δ 5.69, and the two uncoupled aromatic proton singlets at δ 6.43 and 6.56. The remaining signals are complex multiplets, the interpretation of which requires extensive decoupling studies. They account for eight protons consisting of two independent systems H_a, H_b, H_d, and H_f on the B ring and H_c, H_e, and two H_g on the C ring. H_a and H_b are given by two multiplets at δ 1.85 and 1.95, H_d is a doublet multiplet at δ 2.72 and 2.85, while H_f is centered at about δ 3.20. The following coupling constants in hertz for this system were determined: J_{af} = 8.4, J_{ad} = 2.2, J_{bd} = 5.0, J_{bf} = 10.9, and J_{df} = 16.9. The C-ring system is relatively simpler since the ether methylene protons are magnetically equivalent and since there is no coupling between H_c and H_g. The chemical shifts and coupling constants are as follows: H_c at δ 2.15, H_e at δ 3.05, and H_g at δ 3.90 with J_{ce} = 11.2 and J_{ge} = 10 Hz.

As Scheme III illustrates, I gives two products when treated with *tert*-butyl hypochloride as described by Ke-

Scheme III
Chlorohydrin and Epoxide Derivatives of I

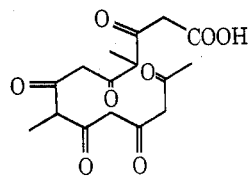


berle and Karrer.[13] The two crystalline products XII and XIII are chlorohydrins with the same molecular formula $C_{16}H_{23}O_4Cl$. The chloronium ion attacks the most electrophilic double bond and the resultant intermediate may be approached from above or below by the hydroxyl group. Since the partial positive charge of the transition state is most likely to be stabilized at the allylic position, the point of OH attachment is almost certainly the C_5 position. Compound XIII, mp 134°, has no carbonyl absorption in the ir spectrum, which indicates that one of the hydroxyl groups is in good field for hemiketal formation. Dreiding models show that the chlorhydrin which results from OH attack from below has two hydroxyl groups in good position for possible hemiketal formation. Compound XIII was isolated unchanged from refluxing acetic anhydride–pyridine solvent, conditions which normally acetylate a free secondary hydroxyl group. On this basis we conclude that the C_1 alcohol is involved in the hemiketal formation. Compound XIV, mp 172–174°, shows strong carbonyl absorption in the ir at 1695 cm^{-1}. The ^1H NMR spectra of both compounds show a split methyl signal at about δ 1.0 and a tertiary methyl signal at δ 1.32. The signal for the other tertiary methyl is at δ 1.2 in XIV but at δ 1.04 in XIII because here carbonyl deshielding is no longer operative. The spectrum of XIII has an exchangeable signal at δ 3.2 for the hemiketal OH and a very sharp exchangeable singlet at δ 5.42 for the proton of the tertiary OH at C_5. In the spectrum of XIV there are analogous exchangeable signals at δ 3.17 and 5.10. The signals between δ 1.5 and 4.2 are complex but similar in both compounds. It is in the olefinic area that the sharp diagnostic differences are observed. Both olefinic protons

of XIII are represented as a sharp singlet at δ 5.64 so they must be virtually magnetically equivalent, a circumstance which can only occur with an α hydroxyl group at C_5 as shown. In XIV these protons are shown as an AB pair as in I at δ 5.23 and 6.12 (J = 10 Hz).

Treatment of I with *m*-chloroperbenzoic acid yields a crystalline epoxide XV. The CO group in this compound is no longer chelated to the C_1 hydroxyl group since it is observed at 1708 cm^{-1} in the ir as opposed to 1680 cm^{-1} in the ir of I. Instead of the epoxide one might visualize the alternate structure XVa. This can be ruled out on the basis of mass spectral data because of the absence of a peak at *m/e* 84. The expected excision of a 3-methyl tetrahydrofuranyl fragment from XVa would give rise to *m/e* 84. In addition the proton of the tertiary OH at C_5 in both chlorohydrins is observed as a sharp singlet between δ 5.0 and 5.5 where there is every reason to expect it would also appear in the spectrum of XVa. The curve of XV shows the vinyl AB pair at δ 5.45 and 6.23 and is lacking any other signal in that area.

The biosynthetic polyketide sequence leading to the first six metabolites of *Sporormia affinis* has been mentioned briefly.[2] The structure I may be formally accommodated to the polyketide shown, which consists of five acetate and



two propionate units. Tertiary methyls such as those at C_8 and C_9 are rare in polyketide-derived material. The plant product portentol contains a tertiary methyl and is said to be formally derived from a polypropionate.[14] Although the authors do not mention it, diplodiatoxin would appear to be of polyketide origin even though it contains a tertiary methyl group.[15] Diplodiatoxin and LL-N313ζ are both fungal metabolites and Turner[16] has pointed out that no fungal product incorporates propionate within the chain but rather tertiary and secondary methyls are introduced directly from the one-carbon pool.

Experimental Section

TLC was carried out on Brinkmann plates. ^1H NMR spectra were run on Varian A-60 or A-100 instruments. Mass spectra were made on an AEI MS9 high-resolution, direct-inlet mass spectrometer. Ir and uv spectra were run on Infracord and Cary 11 spectrophotometers, respectively. A Cary 60 spectropolarimeter was used for CD (2.1 mg/ml of I in MeOH, cell width 0.1 mm) and ORD (1.27 mg/ml of I in MeOH, cell width 0.2 mm) work. Solvents and solutions were dried over anhydrous $MgSO_4$. Melting points were taken by a capillary tube method and are uncorrected.

Isolation of I. Sporormia affinis Sacc., Bomm and Rouss (Lederle culture N313) was incubated for 5 days as previously described.[1] The metabolites were recovered from the filtered beer by batch adsorption on carbon. The carbon was eluted with acidic aqueous acetone and the eluate concentrated to the aqueous phase and extracted with $CHCl_3$. The dried, concentrated $CHCl_3$ extract was chromatographed over silica gel using 1:1 $CHCl_3$–hexane to obtain crude I which could be recrystallized from EtOAc–hexane to get pure I, mp 173–173.5°. Yields varied from 1 to 3 g per 300-l. fermentation: $[\alpha]^{25}D$ −113 ± 2° (*c* 1.05, MeOH); uv max (MeOH) λ 242 nm (ε 19900); ir (KBr) 3440, 1680 cm^{-1}; ^1H NMR (see text); mass spectrum *m/e* 262 (M^+), 245, 244, 201, 172, 157, 146.

Anal. Calcd for $C_{16}H_{22}O_3$: C, 73.25; H, 8.45. Found: C, 73.26; H, 8.19.

The acetate of I was prepared by refluxing in acetic anhydride–pyridine solution for 1 hr. The product was recrystallized from ether–hexane: mp 114.5–115°; $[\alpha]^{25}D$ −49.8 ± 2° (*c* 1.02, MeOH); uv max (MeOH) λ 241 nm (ε 17940); ir (KBr) 1735, 1703, 1385, 1245, 1180, 1095, 945, 880cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00 (3 H, s, 8 CH_3), 1.03 (3 H, d, J = 7 Hz, 3 CH_3), 1.25 (3 H, s, 9 CH_3), 5.03 (1 H, m, 1 CHOAc), 5.47 (1 H, d, J = 10.0 Hz, 6 CH), 5.72 (1 H, br s, 4 CH), 6.22 (1 H, d, 6 CH).

Anal. Calcd for $C_{18}H_{24}O_4$: C, 71.02; H, 7.95. Found: C, 70.70; H, 7.84.

Preparation of II. About 900 mg of I was reduced with 1.35 g of $NaBH_4$ in 20 ml of EtOH. The product was purified by silica gel chromatography and recrystallized from EtOAc to get II: mp 130–131°; $[\alpha]^{25}D$ +79 ± 2 ° (*c* 1.02, MeOH); uv max (MeOH) λ 239 nm ε 20570), sl sh 232 (17900), 247 (14700); ir (KBr) 3450, 3330, no CO peak, weak peaks 1645, 1617 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.0 (3 H, d, J = 7.0 Hz, 3 CH_3), 1.1 (3 H, s, 9 CH_3), 1.25 (3 H, s, 8 CH_3), 5.20 (1 H, d, J = 9 Hz, 7 CH), 5.62 (1 H, s, 4 CH), 6.03 (1 H, d, J = 9.0 Hz, 6 CH).

Anal. Calcd for $C_{16}H_{24}O_3$: C, 72.63; H, 9.15. Found;: C, 72.23; H, 9.11.

1,2,6-Trimethylnaphthalene (III). About 400 mg of I was mixed with 1.0 g of 5% Pd/C and heated for 2 hr to a temperature of 280°. Purification of the resultant oil over alumina yielded 80 mg of III: mp of picrate 121–122° (lit.[17] 121–122°), styphnate 148° (lit.[17] 148°); uv max (hexane) λ 327 nm (ε 1870), 320 (1090), 313 (1530), 290 sh (4670), 282 (5610), 278 (5610), curve matches that of 1,2,6-trimethylnaphthalene;[18] ^1H NMR ($CDCl_3$) δ 2.37 (3 H, aromatic CH_3), 242 (6 H, s, 2 aromatic CH_3), 7.24 (4 H, m, aromatic H), 7.5 (1 H, d, aromatic H).

Approximately 37 mg of III was obtained by chromatographic purification over alumina of the product from overnight refluxing of 500 mg of I in 15 ml of 2:1 dioxane–H_2O solution with 600 mg of *p*-toluenesulfonic acid added: mp of styphnate 148°, picrate 121°; uv curve identical with that mentioned above.

Conversion of II to IV. Approximately 250 mg of II was dissolved in 20 ml of dry MeOH and dry HCl gas passed into the solution until saturated. The solvent was then evaporated and the resultant oil was purified over alumina using hexane solvent to get 120 mg of pure IV: $[\alpha]^{25}D$ +183 ± 1.0° (*c* 0.82, MeOH); uv max (MeOH) λ 267 nm (ε 12000), 223 (33500); ir (KBr) 3000, 1460, 1260, 820, 785 cm^{-1}; ^1H NMR discussed in text; mass spectrum *m/e* 172 (M^+).

Anal. Calcd for $C_{13}H_{16}$: C, 90.64; H, 9.36. Found: C, 90.23; H, 9.40.

Oxidation of I to V. About 500 mg of I in 50 ml of ether was stirred overnight with 1.5 g of $K_2Cr_2O_7$ in 0.3 ml of concentrated H_2SO_4 and 7 ml of H_2O. The product was purified by silica gel chromatography using hexane–EtOAc. Recrystallization of V from EtOAc–hexane yielded 180 mg: mp 159–160°; $[\alpha]^{25}D$ −63.3 ± 3° (*c* 1.03, MeOH); uv max (MeOH) λ 238 nm (ε 36000); ir (KBr) 3000, 1710 (very intense), 1430, 1410, 1360, 1225, 1180, 1090, 1075, 865 (very intense), 785 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.81 (3 H, s, CH_3), 1.12 (3 H, d, J = 7.0 Hz, 3 CH_3), 1.22 (3 H, s, CH_3), 5.52 (1 H, d, J = 10.0 Hz, 7 CH), 5.94 (1 H, m, 4 CH), 6.24 (1 H, d, 6 CH).

Anal. Calcd for $C_{16}H_{20}O_3$: C, 73.82; H, 7.74. Found: C, 73.67; H, 7.82.

Conversion of V to VI. A 200-mg sample of V was dissolved in 1.5 ml of acetone and 1 ml of 4 *N* NaOH was added. The suspension darkened rapidly and after 5 min the reaction mixture was diluted with 10 ml of H_2O and extracted with EtOAc. The solvent extract was dried and evaporated to a gum which was passed over silica gel using 20% EtOAc in hexane solvent. The major component was recrystallized from ether–hexane to yield 110 mg of VI: mp 136–137°; $[\alpha]^{25}D$ +114 ± 1° (*c* 0.85, MeOH); uv max (MeOH) λ 300 nm (ε 7100), (MeOH and NaOH) 305 (9600); ir (KBr) 3000, 2865, 1718, 1665, 1570, 1300, 1080, 830, 795 cm^{-1}; ^1H NMR discussed in text; mass spectrum *m/e* 260 (M^+), 245, 217, 204, 190, 189, 188, 173, 172, metastables 187.7*, 159,3*.

Anal. Calcd for $C_{16}H_{20}O_3$: C, 73.82; H, 7.74. Found: C, 73.26; H, 8.19.

Preparation of VII. About 100 mg of VI were intimately mixed with 200 mg of 10% Pd/C and placed in a sublimator equipped with a cold finger and a collector vial attachment. The apparatus was evacuated to 55 μ and then heated to 250° for 5 min using a Wood's metal bath. After cooling, the vial contained 70 mg of faintly yellow oil which was purified by passage over 7 g of Woelm alumina using 5% EtOAc in hexane solvent to obtain 31 mg of pure VII: uv max (MeOH) λ 247 nm (ε 8200), 298 (2050); ir (KBr) 3000, 1690, 1600, 1565, 1255, 1015, 810 cm^{-1}; ^1H NMR discussed in text; mass spectrum *m/e* 188 (M^+).

Upon refluxing 150 mg of V in 5 ml of MeOH with 0.2 ml of concentrated H_2SO_4 for 1 hr followed by purification of the product by alumina chromatography, a yield of 66 mg of pure VII was obtained.

Conversion of I to VIII. About 25 ml of dry MeOH was satu-

rated with dry HCl gas and then 500 mg of I was added and the resultant solution refluxed for 4 hr. During reflux the condenser was protected from moisture. The solvent was evaporated and the resultant oil taken up in $CHCl_3$ and extracted with bicarbonate solution. The extracted $CHCl_3$ solution was dried and concentrated to 480 mg of an oil which was purified by partition chromatography over diatomaceous earth using a heptane saturated with CH_3CN system. The main product consisted of 250 mg of a colorless oil labeled VIII: $[\alpha]$ ^{25}D −83.5 ± 3.0° (c 1.10, MeOH); ir (KBr) 3000, 2920, 1705, 1485, 1445, 1375, 1112, 812 cm^{-1}; ^1H NMR discussed in text.

Anal. Calcd for $C_{17}H_{22}O_2$: C, 79.07; H, 8.52. Found: C, 79.22; H, 8.08.

Conversion of I to IX. A sample consisting of 500 mg of I was refluxed for 4 hr in 20 ml of dioxane with 7 ml of 4 *N* HCl added. Recovery of 180 mg of product IX was handled as described under preparation of VIII: $[\alpha]$ ^{25}D −78.5 ± 2.0° (c 0.43, MeOH); uv max (MeOH) λ 264 nm (ε 6600), 222 (23000); ir (KBr) 3000, 2930, 1705, 1605, 1485, 1445, 1380, 1290, 1075, 1050, 990, 890, 815, 780, 730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.30 (3 H, d, *J* = 7.5 Hz, 8 CH_3), 1.33 (3 H, s, 9 CH_3), 2.34 (3 H, s, 3 CH_3), 3.6 (2 H, m, 13 CH_2), 5.90 (1 H, dd, *J* = 9.5 and 5.5 Hz, 7 CH), 6.33 (1 H, d, *J* = 9.5 Hz, 6 CH), 6.90 (1 H, s, aromatic H), 7.06 (1 H, s, aromatic H), 7.10 (1 H, s, aromatic H).

Anal. Calcd for $C_{16}H_{19}OCl$: C, 73.16; H, 7.23; Cl, 13.14. Found: C, 73.24; H, 7.51; Cl, 12.94.

Conversion of I to X and XI. An oil suspension of NaH was washed with hexane and resuspended in dry THF to provide an approximately 1 M suspension. About 400 mg of I in 5 ml of dry THF was treated with 1.5 ml of the NaH suspension in THF and 1.5 ml of CH_3I and the reaction mixture was stirred for 2 hr at dry ice–MeOH temperature. The reaction was run under positive N_2 pressure and protected from moisture. The reaction mixture was then partitioned between EtOAc and H_2O. The EtOAc solution was dried and evaporated to a solid which was resolved by silica gel chromatography using a gradient of 5–10% EtOAc in hexane. Two products were recovered. The first product to come off the column consisted of 180 mg of material which was recrystallized from EtOAc–hexane to yield XI: mp 117–119°; $[\alpha]$ ^{25}D −18.9 ± 2° (c 0.50, MeOH); uv max (MeOH) λ 240 nm (ε 26100); ir (KBr) 3650, sh at 3500, 3000, 1680, 1465, 1390, 1300, 1225, 1175, 1110, 1080, 1045, 990, 980, 885, 863, 812, 755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.99 (3 H, s, 12 CH_3), 1.00 (3 H, d, *J* = 7 Hz, 3 CH_3), 1.01 (3 H, s, 12 CH_3), 1.28 (3 H, s, CH_3), 1.36 (3 H, s, CH_3), 3.56 (1 H, d, *J* = 12 Hz, 13 CH), 3.78 (1 H, d, *J* = 12 Hz, 13 CH), 3.80 (1 H, br s, 1 CH), 5.27 (1 H, d, *J* = 10 Hz, 7 CH), 5.64 (1 H, s, 4 CH), 6.05 (1 H, d, *J* = 10 Hz, 6 CH); mass spectrum *m/e* 290 (M^+), 272, 257, 243, 229, 199, 188, 173, 172, 171.

Anal. Calcd for $C_{18}H_{26}O_3$: C, 74.44; H, 9.03. Found: C, 74.31; H, 8.61.

The second material off the column amounted to 60 mg which upon recrystallization from EtOAc–Hexane yielded 47 mg of X: mp 144–145°; $[\alpha]$ ^{25}D −111.8 ± 2.0° (c 0.50, MeOH); uv max (MeOH) λ 242 nm (ε 22100); ir (KBr) 3650, 3500, 3000, 2900, 1700, 1460, 1375, 1315, 1240, 1180, 1115, 1075, 1045, 990, 980, 875, 868, 840, 805, 790, 755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00 (3 H, d, *J* = 7 Hz, 3 CH_3), 1.01 (3 H, s, CH_3), 1.04 (3 H, d, *J* = 7 Hz, 3 CH_3), 1.19 (3 H, s, CH_3), 3.46 (1 H, t, *J* = 11.5 Hz, 13 CH), 3.80 (1 H, br s, 1 CH), 3.95 (1 H, m, *J* = 10 Hz, 6 CH); mass spectrum *m/e* 276 (M^+), 258, 243, 215, 188, 173, 172, 171.

Anal. Calcd for $C_{17}H_{24}O_3$: C, 73.88; H, 8.75. Found: C, 73.64; H, 8.82.

Conversion of I to XII. Approximately 400 mg of I together with 500 mg of 30% Pd/C were refluxed for 2 hr in 10 ml of *n*-dihexyl ether.[13] The product was purified by silica gel chromatography using a gradient of 0–5% EtOAc in hexane. About 120 mg of a white solid was recovered which when recrystallized from EtOAc–hexane yielded XII: mp 227–228°; $[\alpha]$ ^{25}D −34.5 ± 0.3° (c 0.66, MeOH); uv max (MeOH) λ 284 nm (ε 1800), 278 (1700), 215 (5400); (MeOH + NaOH) 293 (3000); ir (KBr) 3360, 2940, 1700, 1628, 1595, 1435, 1390, 1380, 1335, 1255, 1112, 1068, 1028, 845 cm^{-1}; ^1H NMR discussed in text.

Anal. Calcd for $C_{16}H_{20}O_3$: C, 73.82; H, 7.74. Found: C, 73.68; H, 7.62.

Formation of Chlorohydrins XIII and XIV. A sample of 500 mg of I was dissolved in 0.5 ml of acetone and 0.5 ml of H_2O was added followed by 0.15 ml of *t*-BuOCl. The mixture was stirred at room temperature for 1 hr. The products were purified by silica gel column chromatography using a gradient of 5–10% EtOAc in hexane. The first product off the column upon recrystallization from EtOAc–hexane yielded 80 mg of XIII: mp 134°; $1\alpha]$ ^{25}D +65.0 ± 0.4° (c 0.57, MeOH); ir (KBr) 3400, 2930, 1455, 1430, 1382, 1367, 1345, 1275, 1255, 1200, 843, 823, 706 cm^{-1}; ^1H NMR ($CDCl_3$) 1.01 (3 H, d, *J* = 7.5 Hz, 3 CH_3), 1.04 (3 H, s, 9 CH_3), 1.32 (3 H, s, 8 CH_3), 5.22 (1 H, d, *J* = 10 Hz, 7 CH), 5.42 (1 H, s, (exchangeable, 5 COH), 6.12 (1 H, d, *J* = 10 Hz, 6 CH); mass spectrum *m/e* 314 (M^+), 281, 263, 245, 227, 189, 171.

Anal. Calcd for $C_{16}H_{23}O_4Cl$: C, 61.04; H, 7.31; Cl, 11.28. Found: C, 61.40; H, 7.35; Cl, 11.11

The second product consisted of 70 mg of XIV: mp 172–174°; $[\alpha]$ ^{25}D −110 ± 0.4° (c 0.53, MeOH); ir (KBr) 3430, 2930, 2980, 2935, 1695, 1420, 1390, 1380, 1310, 870, 843, 795, 785, 750 cm^{-1}; ^1H NMR ($CDCl_3$) 1.03 (3 H, d, *J* = 7.5 Hz, 3 CH_3), 1.20 (3 H, s, 9 CH_3), 1.32 (3 H, s, 8 CH_3), 5.10 (1 H, s, exchangeable, 5 COH), 5.64 (2 H, s, 6 CH and 7 CH).

Anal. Calcd for $C_{16}H_{23}O_4Cl$: C, 61.04; H, 7.31. Found: C, 60.84; H, 7.33.

Formation of Epoxide XV. A solution of 140 mg of I in 10 ml of CH_2Cl_2 with 100 mg of *m*-chloroperbenzoic acid was refluxed overnight. The reaction solution was then washed with dilute $NaHCO_3$ solution, dried, and evaporated to dryness to yield a white solid which was recrystallized from ether–hexane to yield a first crop of 50 mg of XV: mp 121–122°; $[\alpha]$ ^{25}D −45.7 ± 2.0° (c 1.03, MeOH); ir (KBr) 3500, 3000, 1708, 1460, 1420, 1375, 1110, 925, 870, 795 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.14 (3 H, d, *J* = 7.5 Hz, 3 CH_3), 1.29 (3 H, s, CH_3), 1.31 (3 H, s, CH_3), 8.45 (1 H, d, *J* = 10 Hz, 7 CH), 6.23 (1 H, d, *J* = 10 Hz, 6 CH); mass spectrum *m/e* 278 (M^+), 263, 260, 250, 245, 236, 231.

Anal. Calcd for $C_{16}H_{22}O_4$: C, 69.04; H, 7.97. Found: C, 68.80; H, 8.10.

Configuration of C_1 in I by Horeau Method. A sample of 70 mg of I was dissolved in 3 ml of pyridine, 0.3 ml of DL-α-phenylbutyric anhydride was added, and the reaction was allowed to proceed overnight. Work-up of the reaction in the standard manner yielded 200 mg of crystalline α-phenylbutyric acid (TLC, ir, and C, H analysis matched data of authentic material) which had $[\alpha]$ ^{25}D −1.85 ± 0.22° (c 4.45, benzene). The excess of (−) or *R* acid indicates that C_1 is *S*.

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Registry No.—I, 53342-17-9; I acetate, 57016-66-7; II, 57016-67-8; III picrate, 57016-68-9; III styphnate, 57016-69-0; IV, 57016-70-3; V, 57016-71-4; VI, 57016-72-5; VII, 57016-73-6; VIII, 57016-74-7; IX, 57016-75-8; X, 57016-76-9; XI, 57016-77-0; XII, 57016-78-1; XII, 57016-78-1; XII, 57016-79-2; XIV, 57049-20-4; XV, 57016-80-5.

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Carbon-13 Nuclear Magnetic Resonance Spectra of Hydroxy Steroids

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^{13}C NMR spectra have been obtained and the individual resonances assigned for 31 monohydroxylated androstanes and cholestanes as well as a number of acetoxy derivatives. The chemical shifts are rationalized in terms of α, β, γ, and δ substituent effects. The variation of these effects is discussed in terms of steric interactions of the hydroxyl group. Quantitative correlations are presented relating the α and β substituent effects to the type and number of specific steric interactions of the hydroxyl group. These correlations allow the estimation of substituent shifts of α- and β-carbon atom resonances within 2.0 and 1.0 ppm, respectively. The magnitude of the γ-gauche shift is correlated with 1,3-syn-diaxial OH–CH_3 interactions; furthermore, the possible dependence of the γ-gauche shift upon the presence of a proximate hydrogen atom at the γ carbon is discussed. The downfield δ substituent effect found with OH–C(δ) skew pentane configurations is rationalized in terms of steric deformations to relieve the interaction.

Until very recently the literature data regarding ^{13}C nuclear magnetic resonance spectra of steroids have been of a somewhat fragmentary nature. Therefore we have undertaken a systematic investigation of these compounds by ^{13}C NMR in order to determine (and develop a predictive rationale to describe) the influence of substituents, position, and stereochemistry on their spectra. In the present study we describe the ^{13}C NMR spectra of a series of hydroxy steroids. A previous paper has dealt with the spectra of keto steroids[2] and a study of monounsaturated steroids will be presented in a forthcoming paper.[3] Complete assignments in these series of monofunctional steroids is an absolute prerequisite before attempting the interpretation and eventual prediction of the ^{13}C NMR spectra of polyfunctional steroids, which are frequently of great biological interest. Furthermore, because of the skeletal rigidity which precludes the possible complication of conformational interconversions, hydroxy substituted steroids (such as androstanols and cholestanols) provide an ideal material in which to study the influence of geometrical and stereochemical features upon the substituent effects of the hydroxyl group in cyclic systems. Nearly all possible geometrical environments of the hydroxyl group are represented by the 31 examples in this series, which includes 11 different epimeric pairs.

Experimental Section

The hydroxy steroids (see Table I) included in this study are all known compounds and have been prepared by the following methods: **2** by lithium aluminum hydride reduction,[4] and **4** by reduction with sodium in ethanol[5,6] of cholestan-1-one;[7] **7** by lithium aluminum hydride reduction[8] of cholestan-2-one;[7] **13** by hydroboration–oxidation[9] of Δ^4-cholestene; **15** by Jones oxidation of **13** followed by lithium aluminum hydride reduction;[10] **16** by epoxidation of Δ^4-cholestene[11] followed by lithium aluminum hydride reduction;[12] **17** by reduction with sodium in ethanol[4] of cholestan-6-one[4] and **18** by lithium aluminum hydride reduction[8] of androstan-6-one;[13] **21** by reduction with lithium in ammonia,[15] and **22** by lithium aluminum hydride reduction[8] of androstan-11-one;[14] **23** was prepared analogous to **24** (vide infra) from 12α-acetoxy-5α-spirostane; **26** by hydroboration–oxidation of Δ^{14}-cholestene;[17] **29** by lithium aluminum hydride reduction[8] of androstan-16-one.[9] The following compounds were prepared by previously described methods: **1**,[18] **3**,[18] **19**,[4] **20**,[4] **24**,[16] **27**.[19] An attempt to prepare **3** by reduction of 1-androstanone with K(*sec*-Bu)$_3$BH ("K-selectride", Aldrich)[20] gave only a very small amount (~5%) of the desired product, most of the starting material being recovered unchanged. Compounds **5, 6, 12, 14, 25, 28,** and **30** were generously provided by Dr. Paul V. Demarco.[21]

All the acetoxy steroids were prepared by reaction of the alcohol with acetic anhydride in pyridine, with the exception of 5α-acetoxycholestane, which was made according to the procedure of Plattner et al.[22]

The ^{13}C NMR spectra were recorded at 25.2 MHz using a Varian XL-100-15 system or at 22.6 MHz with a Bruker WH-90 spectrometer, both operating in the Fourier transform mode. Data were accumulated with a maximum of 1.2 Hz per data point. The chemical shifts are relative to internal Me_4Si and are estimated to be accurate to ±0.1 ppm. The probe temperature was ca. 30°.

The spectra were determined as 0.2–0.6 *M* solutions in $CDCl_3$. Variation in sample concentration was found to have a negligible influence (less than 0.1 ppm) on the chemical shift values of all carbons except the carbinyl carbon atom. With increasing sample concentration, within the employed range, this carbon atom became increasingly shielded by up to 0.3 ppm.

The shift reagent experiments were performed with commercial Eu(dpm)$_3$ or Eu(fod)$_3$, which were used without further purification. The ^{13}C spectra were first recorded in the proton noise-decoupling mode in order to measure the exact chemical shifts of all the ^{13}C nuclei present. The degree of substitution of each carbon atom was determined by obtaining a second series of spectra in the single-frequency off-center decoupling mode. Subsequently, a freshly prepared solution of shift reagent in $CDCl_3$ was added in two equal increments to each sample solution and the spectral data in the two modes redetermined. The final molar ratio of reagent to steroid was 0.3. The effects of the addition of the shift reagent on the chemical shift of the ^{13}C nuclei appeared linear in this range.

Results

Chemical shift data for the hydroxy steroids examined are given in Table I along with the values for the parent hydrocarbons, androstane and cholestane. ^{13}C NMR data for