# Nuclear Magnetic Resonance Spectroscopy. Carbon-13 Spectra of Cholic Acids and Hydrocarbons Included in Sodium Desoxycholate Solutions[1a]

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**Abstract:** The $^{13}C$ resonances of cholic, desoxycholic, chenodesoxycholic, and lithocholic acids and some of their derivatives have been recorded and assigned to specific carbons. Comparison of the carbon chemical shifts for steroids with cis and trans A/B ring junctions show substantial similarities for the atoms in the C and D rings but large differences for some of the atoms in the A and B rings. The task of making the assignments was facilitated by comparisons of the $T_1$ relaxation times of the carbon nuclei and the use of lanthanide shift reagents to separate the carbon resonances sufficiently to simplify the interpretation of the off-resonance, partially proton-decoupled, spectra. Sodium desoxycholate in $D_2O$ solution gives rather broad carbon resonances for all but the methyl and the quaternary carbons, as expected for substantial intermolecular association. These solutions take up *p*-xylene and 2-methylnaphthalene in about 2:1 molar ratios. The $^{13}C$ spectra of the substances so included indicate that there is considerable restriction of the molecular motion of the 2-methylnaphthalene, but relatively little of *p*-xylene.

The recent rapid development of nmr spectrometers using pulsed rf power and Fourier transform analysis of free-induction decay spectra has permitted taking the $^{13}C$ magnetic resonance (cmr) spectra at natural-abundance levels of even quite complex natural products at low concentrations in rather short times. However, obtaining a cmr spectrum of such compounds and interpreting it are not quite the same thing. The steroids present an especially difficult problem because many of their resonances fall in a fairly narrow shift range. An early study of all-trans steroids[2] was greatly facilitated by the plethora of available substitution products which permitted reasonable assignments of the carbon resonances in terms of the known effects of substitution on conformationally fixed cyclohexane rings. The steroids with cis A/B ring fusions are not as readily available with single changes in substitution and, in the work to be described here mainly with the bile acids (**1–4**), it was necessary to use additional techniques to assign the resonances to specific carbons.

## Experimental Section

The steroids used in this work were mostly recrystallized commercial materials. Methyl esters were prepared by treatment of the corresponding acids with diazomethane, while acetates were obtained by treatment with acetic anhydride. Reduction of the methyl esters with lithium aluminum hydride gave the C-24 alcohols corresponding to the various bile acids.

Chemical shifts were measured with a "Brukarian" pulsed FT spectrometer which was the previously described[3] Varian digital frequency sweep spectrometer operating at 15.09 MHz, but modified by substitution of a Bruker pulse amplifier, probe, receiver, and internal deuterium lock. The pulses were derived from the Varian pulse box, and the free induction decay was accumulated and transformed with a 16K Varian 620i computer. The difficult task of interfacing these supposedly incompatible components with the one-of-a-kind DFS spectrometer was carried through by Dr. Bruce L. Hawkins with valuable help in the initial stages of Dr. J.-Y. Lallemand. Some spectra were obtained at 25.2 MHz with an FT-equipped Varian XL-100 spectrometer at the laboratories of Varian Associates, and 55.3-MHz spectra were obtained with a FT-equipped Varian HR 220 instrument.

The inversion–recovery method[4] was used for the measurement of the $T_1$ values of the carbon nuclei. The steroids were studied in $CH_2Cl_2$, $CH_2Cl_2$–$CDCl_3$, or $CH_2Cl_2$–dioxane mixtures at 0.5–1.0 *M* concentrations, except for a few cases where solubilities or quantities available permitted only 0.1–0.2 *M* concentrations. The shifts were referenced to $CH_2Cl_2$ as internal standard and converted to the $CS_2$ scale by the relation $\delta_{CS_2} = \delta_{CH_2Cl_2} + 138.8$ ppm. The lanthanide-induced chemical shift changes were measured relative to $CH_2Cl_2$ associated with several successive 0.05 *M* increments in the lanthanide concentration. The lanthanide shifts are calculated for 1:1 molar ratios of chelate to steroid. The steroids could usually be recovered easily from solutions containing the lanthanide chelates by column chromatography on silica gel. The chelate can be removed with methylene chloride and the steroid eluted with ether.

## Results and Discussion

For assigning the cmr resonances to specific carbons in complex spectra, it is imperative to know how many hydrogens are attached to each carbon. The most straightforward way of doing this is by partial single-frequency off-resonance (sfor) proton decoupling,[2,5] but with molecules such as steroids, where many of the carbon shifts fall in a fairly narrow range, the overlap of the sfor lines is often so great as to preclude positive identification of the splitting patterns. Where applicable, as with most of the compounds studied here, lanthanide shifts can be extremely useful to separate sfor lines to determine their multiplicities; the lanthanide chelate having no effect on the magnitude of the couplings of the carbons with the directly attached hydrogens. We have found it convenient when investigating the sfor lines to add successive portions of europium trisdipivaloylmethane, Eu(dpm)$_3$, for downfield shifts,[6a] or praseodymium tris(1,1,1,2,2,3,3-hepta-

(1) (a) Supported by the Public Health Service, Research Grant No. GM-11072 from the Division of General Medical Sciences, and by the National Science Foundation. (b) Deutsche Forschungsgemeinschaft Postdoctoral Research Fellow, 1970–1971.

(2) H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert, and J. D. Roberts, *J. Amer. Chem. Soc.*, **91**, 7445 (1969).

(3) (a) F. J. Weigert and J. D. Roberts, *ibid.*, **89**, 2967 (1967); (b) F. J. Weigert, M. Jautelat, and J. D. Roberts, *Proc. Nat. Acad. Sci. U. S.*, 60, 1152 (1968).

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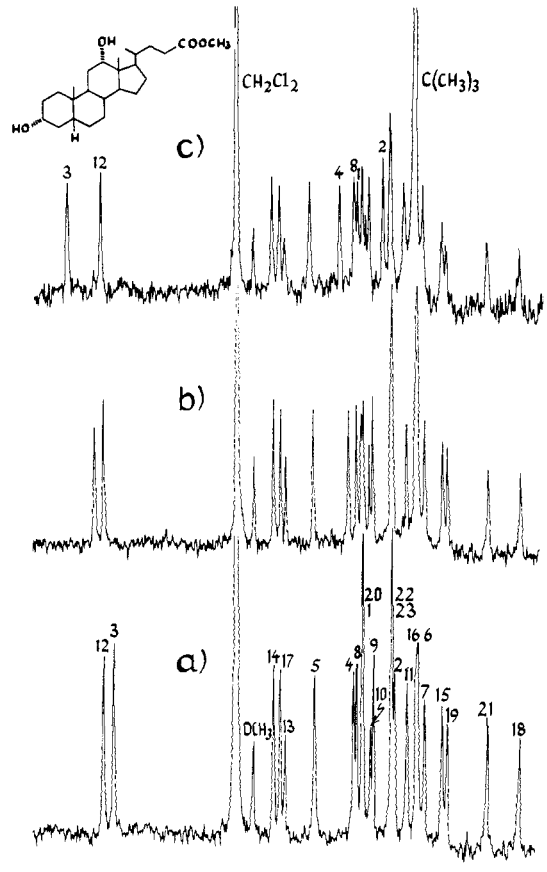


Figure 1. Changes in the $^{13}C$ spectrum at 15.09 MHz of methyl desoxycholate (**2a**) in methylene chloride on addition of successive increments of $Eu(dpm)_3$. The highest concentration of $Eu(dpm)_3$ is for c with 0.2 mol equiv of chelate/mol of **2a**; a contains no chelate.

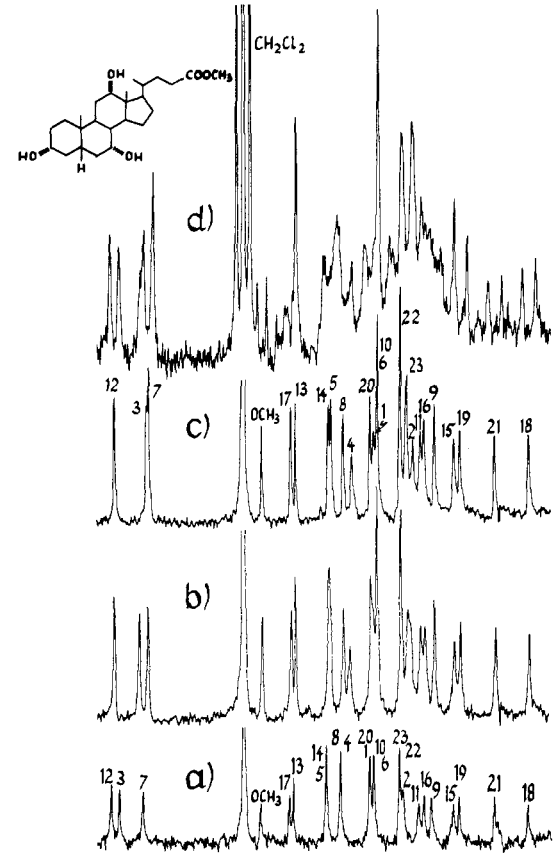


Figure 2. Changes in the $^{13}C$ spectrum at 15.09 MHz of methyl cholate (**4a**) in methylene chloride on addition of successive increments of $Pr(fod)_3$. The highest concentration of $Pr(fod)_3$ is for d with 0.2 mol equiv of chelate/mol of **4a**; a contains no chelate.

fluoro-7,7-dimethyl-4,6-octanedione), $Pr(fod)_3$,[6b] for upfield shifts until the sfor lines of a given carbon are well enough separated from those of its neighbors to have a clearly assignable multiplicity. To avoid interference from the carboxylic acid function, the lanthanide shifts were determined on the methyl esters of the bile acids. These esters give the same chemical shifts for the ring carbons as the acids and generally have better solubility. Figure 1 shows the changes in the $^{13}C$ nmr shifts which occur on adding successively larger amounts of $Eu(dpm)_3$ to solutions of methyl desoxycholate (**2a**). At 0.2 mol equiv of $Eu(dpm)_3$/mol of ester, the resonances, which we will show later to be assignable to C-4, C-8, C-1, C-20, C-9, and C-10, are fairly well separated. It is clear from the shifts that the rather hindered axial hydroxyl at C-12 is less readily complexed than the hydroxyl at C-3, the C-3/C-12 lanthanide shift ratio being 20:1. This is in marked contrast with *cis*- and *trans*-4-*tert*-butylcyclohexanol,[7] where C-1 for the axial cis hydroxyl undergoes markedly larger lanthanide shifts than for the trans alcohol.

If the cmr spectrum is less cluttered on the upfield side of a peak of interest, it may be advantageous to use the praseodymium chelate. Thus, with 0.2 mol of $Pr(fod)_2$/mol of methyl cholate (see Figure 2), the peaks for C-14 and C-5, as well as C-8 and C-4, become separated and the sfor spectrum (Figure 2d) shows three tertiary carbon resonances and one secondary carbon. It will be seen again that the axial hydroxyls at C-7 and C-12 are associated with smaller lanthanide shifts for these carbons than for C-3. Because of differences in the degree of nuclear Overhauser enhancement (NOE), the relative line intensities in proton noise-decoupled cmr spectra may differ widely[8] from expectations based on simple statistics. This situation, which is quite inimical to quantitative analysis, can be relieved by addition of small amounts of paramagnetic material to shorten the proton relaxation times and quench the NOE.[9] Chromium perchlorate has been used for this purpose, but the corresponding acetylacetonate is more soluble in organic solvents.[9] The effect of 0.1 mol equiv of $Cr(AcAc)_3$ on the cmr spectrum of a dilute solution of methyl cholate is shown in Figure 3. In the presence of the chromium chelate, the relative intensities of the resonances are about in the statistical ratios, except for the methyl and quaternary carbons.

The utility of $T_1$ measurements for differentiation of different kinds of carbons with different numbers of protons in large molecules has been well established[10] and can be a useful adjunct to sfor measurements for

(7) D. Leibfritz and J. D. Roberts, unpublished research.

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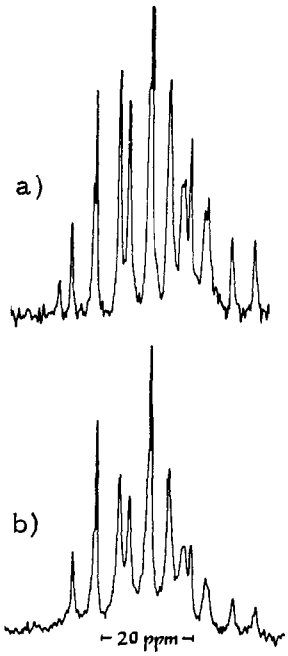
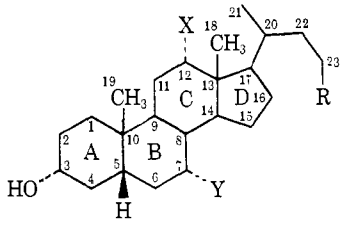



Figure 3. Effect on high-field region of $^{13}C$ spectrum of methyl cholate dissolved in methylene chloride on adding $Cr(AcAc)_3$: (a) no $Cr(AcAc)_3$; (b) 0.1 mol equiv of $Cr(AcAc)_3$.

determining the character of the carbon resonances. Thus, in **2**, the $T_1$ values of the ring methylene carbons turn out to be about 0.25 sec, the ring methine carbons about 0.4 sec, and the quaternary carbons about 2 sec. This pattern of $T_1$ figures is in accord with the conclusion[10] that the dipolar relaxation mechanism predominates for $^{13}C$ in this type of molecule.

**Assignments.** Lithocholic (**1**), desoxycholic (**2**), chenodesoxycholic (**3**), and cholic (**4**) acids served as a basis set for assigning the resonances for the steroids with cis A/B ring junctions.




**1**, lithocholic acid (X = Y = H; R = $CO_2H$); **1a**, R = $CO_2CH_3$
**2**, desoxycholic acid (X = OH; Y = H; R = $CO_2H$); **2a**, R = $CO_2CH_3$; **2b**, R = $CH_2OH$
**3**, chenodesoxycholic acid (X = H; Y = OH; R = $CO_2H$); **3a**, R = $CO_2CH_3$; **3b**, R = $CH_2OH$
**4**, cholic acid (X = OH; Y = OH; R = $CO_2H$); **4a**, R = $CO_2CH_3$

**Assignments for ring A** were made as follows. The C-3 resonance appears in **1–4** at 121.4 ± 0.3 ppm and, being equatorial, is not overlapped by the resonances of C-7 in **3** and **4** or C-12 in **2** or **4**. Formation of the acetate shifts the C-3 signal by −2.7 ± 0.2 ppm to lower field in accord with the −2.9-ppm shift of C-1 observed on acetylation of the equatorial hydroxyl of *trans*-4-*tert*-butylcyclohexanol.[2] The sfor peaks from C-3 are characteristically different from those derived from C-7 and C-12 when these are carrying axial hydroxyls, because the adjacent protons on C-2 and C-4 are strongly coupled by virtue of having the trans diaxial relationship to the C-3 proton. The result is that the C-3 sfor signals are broader than those from

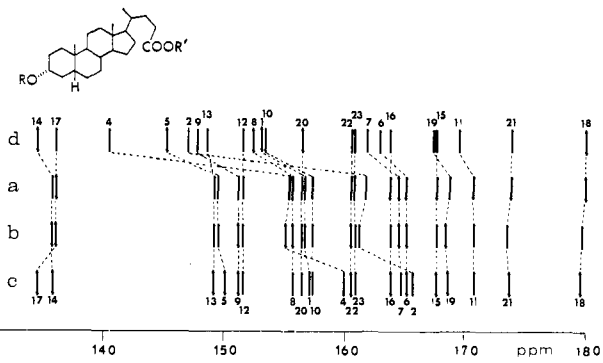
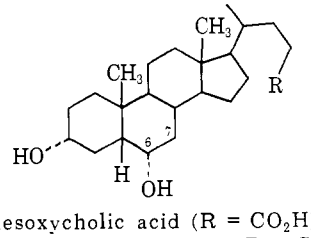



Figure 4. Correlation diagram of $^{13}C$ shifts for lithocholic acid and its derivatives: (a) lithocholic acid (**1**) in methanol; (b) methyl lithocholate (**1a**) in methylene chloride; (*c*) 3-acetate ester of **4a** in dioxane–$CH_2Cl_2$; (d) **4a** with 1.0 mol equiv of $Eu(dpm)_3$ in methylene chloride.

C-7 or C-12. The resonances of C-2 and C-4 may be assigned as the result of their upfield shift on acetylation (see the correlation diagrams shown in Figures 4–7). Differentiation of C-2 and C-4 can be achieved by comparison of **1** and **3**, **1** and **4**, or **2** and **3** (see Figure 8). The C-2 resonance in **1–4** is in substantially the same position, while the C-4 shift is downfield by more than 3 ppm if C-7 carries an axial hydroxyl. This downfield shift is consistent with the shifts observed when a cyclohexane has two 1,3-diaxial methyl groups[11] and is due to the steric effect between the hydrogens on C-4 and the axial hydroxyl on C-7.

The C-1 and C-5 shifts are almost the same in all four compounds and were assigned with the aid of the lanthanide shifts which were the same as observed for *trans*-4-*tert*-butylcyclohexanol.[7] The resonance of the quaternary C-10 is in the same position and can be differentiated from the resonance of the quaternary C-13 by its larger lanthanide shift. Further distinction between C-10 and C-13 is possible by comparison of **1** and **3** with **2** and **4** wherein it will be seen that C-13 is shifted to lower field by ~4 ppm as the result of substitution of C-12 with an axial hydroxyl, this being characteristic of the axial $\beta$ effect in alicyclic alcohols[12] and hydrocarbons.[13]

**The B-ring assignments** were deduced by the following scheme. Differentiation between C-6 and C-7 in **1** and **2** was hampered by their resonances showing similar lanthanide shifts (Figure 4), identical $T_1$ relaxation times (Table I) and negligibly different shifts on acetylation at C-3. The upfield line of this pair was assigned to C-7 on the basis of the cmr spectrum of methyl hyodesoxycholate (**5a**) in which the C-7 resonance




**5**, hyodesoxycholic acid (R = $CO_2H$)
**5a**, methyl hyodesoxycholate (R = $CO_2CH_3$)

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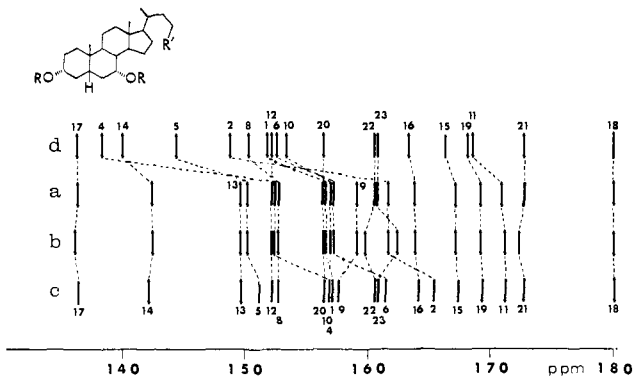



Figure 5. Correlation diagram of $^{13}C$ shifts for chenodesoxycholic acid (**3**) and its derivatives: (a) **3** in methanol; (b) the reduction product (**3b**) in methanol; (c) 3,7-diacetate of methyl chenodesoxycholate (**3a**) in dioxane; (d) **3a** with 1.0 mol equiv of $Eu(dpm)_3$ in methylene chloride.

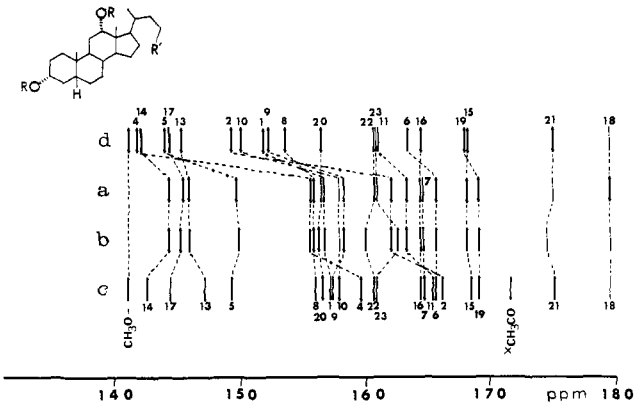



Figure 6. Correlation diagram of $^{13}C$ shifts for desoxycholic acid and its derivatives: (a) desoxycholic acid (**2**) in methanol; (b) methyl desoxycholate (**2a**) in dioxane; (c) 3,12-diacetate ester of **2a** in dioxane; (d) **2a** with 1.0 mol equiv of $Eu(dpm)_3$ in methylene chloride.

appears at 162.2 ppm. The location of this resonance is determined by a $\beta$ shift produced through substitution of an equatorial hydroxyl on a cyclohexane ring.[12] The magnitude of the measured shift is more nearly correct if the C-7 resonances of **1** and **2** come at 166.1–166.2 ppm rather than at 165.2–165.3 ppm (see Figure 8). The ~165.2-ppm shift assigned to C-6 in **1** and **3** is somewhat upfield of the C-6 shifts for all trans steroids, and this is reasonable, there being an equatorial $\beta$ effect of C-4 on C-6 in the trans steroids which becomes an axial $\beta$ effect when the ring junction is cis (compare **6a** and **6b**).[12,13]

The C-8 resonances are characterized as the only tertiary carbon resonances of **1** and **2** which are shifted downfield by introduction of a $\beta$ axial hydroxyl at C-7 to the extent of −3.8 and −3.5 ppm, respectively.

In the all-trans steroids,[2] the C-9 resonance is well separated from the majority of those of the other carbons. However, with the A/B ring junction cis, the C-9 resonance is shifted 14 ppm to higher field. The reason is the twofold interactions of the hydrogen on C-9 with the hydrogens on C-2 and C-4, which are absent in the steroids, with the trans A/B ring junction (compare **6a** and **6b**).[12,13] These interactions produce about a 6–7 ppm upfield shift apiece. In contrast, C-7 has but one of these interactions (with the hydrogen on C-4), and its resonance is shifted upfield by 6 ppm. C-7 is further substituted by an axial hydroxyl, as in **3**, the shift of C-9 is an additional 7.4 ppm to higher field. The corresponding substitution at C-12, as in **2**, produces a 6.8-ppm shift for C-9. Both substitutions, as in **4**, produce a 14-ppm upfield shift for C-9.

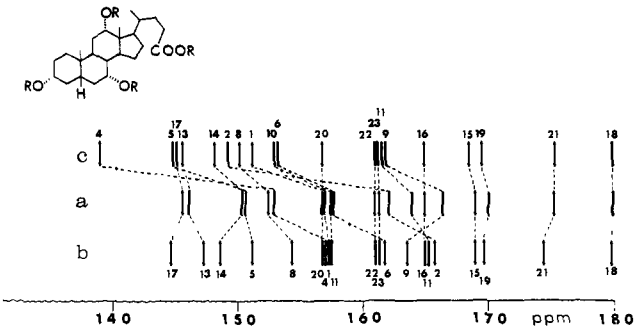



Figure 7. Correlation diagram of $^{13}C$ shifts of cholic acid and its derivatives: (a) cholic acid (**4**) in methanol; (b) 3,7,12-triacetate ester of methyl cholate (**4a**) in dioxane; (c) **4a** with 1.0 mol equiv of $Eu(dpm)_3$ in methylene chloride.

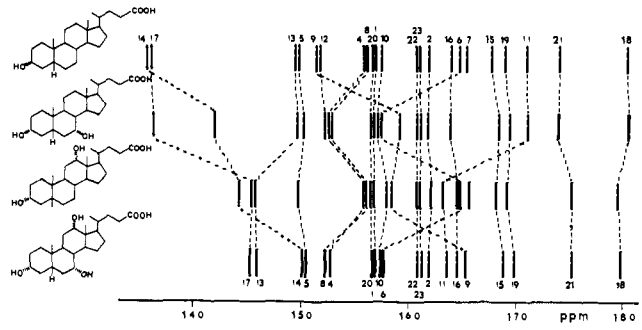
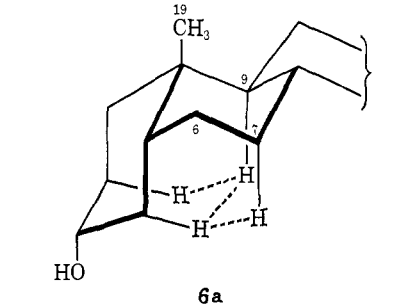
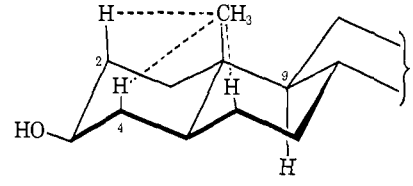



Figure 8. Correlation diagram of $^{13}C$ shifts of **1**, **2**, **3**, and **4** in methanol.




**6a**




**6b**

**The C-ring assignments** are greatly facilitated by comparisons with the corresponding trans steroids because the stereochemistry of the A/B ring junction does not markedly influence the C-ring shifts. The resonance of C-11 is established as that of the $CH_2$ carbon, with the large upfield shift being produced by the axial methyl groups. Substitution of an axial hydroxyl at C-12, as in **2** and **4**, makes for a downfield

**Table I**

| Carbon | Cholanic methyl ester | Lithocholic acid: Acid | Lithocholic acid: Methyl ester | Lithocholic acid: Ester acetate | 5 methyl ester | Chenodesoxycholic acid: Acid | Chenodesoxycholic acid: Methyl ester | Chenodesoxycholic acid: Ester acetate | Chenodesoxycholic acid: Alcohol | Desoxycholic acid: Acid | Desoxycholic acid: $T_1$ | Desoxycholic acid: Methyl ester | Desoxycholic acid: Ester acetate | Desoxycholic acid: Alcohol | Cholic acid: Acid | Cholic acid: Methyl ester | Cholic acid: Ester acetate | 7 | Acetate | Coprostane | Epicoprostanol | Cholestane | Cholestanol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C-1 | 134.9 | 157.2 | 157.2 | 157.7 | 156.5 | 157.0 | 157.0 | 157.5 | 157.0 | 157.1 | 0.28 | 157.1 | 157.5 | 157.1 | 157.1 | 157.1 | 157.4 | 157.1 | 157.4 | 154.9 | 157.0 | 153.6 | 155.4 |
| C-2 | 171.2 | 162.4 | 161.9 | 166.4 | 163.1 | 161.8 | 161.5 | 161.2 | 161.5 | 162.3 | 0.21 | 162.1 | 166.1 | 162.3 | 162.2 | 161.9 | 165.4 | 162.0 | 165.8 | 171.1 | 161.7 | 170.1 | 160.9 |
| C-3 | 165.0 | 121.0 | 120.9 | 118.4 | 121.0 | 121.4 | 120.7 | 118.5 | 120.5 | 121.1 | 0.45 | 121.0 | 118.6 | 121.4 | 121.2 | 120.6 | 118.5 | 121.2 | 118.2 | 165.3 | 120.6 | 165.5 | 121.2 |
| C-4 | 165.4 | 156.3 | 156.0 | 160.6 | 157.5 | 153.1 | 152.9 | 157.6 | 153.1 | 156.7 | 0.23 | 156.4 | 160.6 | 156.5 | 153.2 | 152.9 | 157.3 | 156.1 | 160.2 | 164.9 | 155.8 | 163.6 | 154.1 |
| C-5 | 148.7 | 150.3 | 150.3 | 150.8 | 144.0 | 150.7 | 150.7 | 151.6 | 150.7 | 150.1 | 0.38 | 150.2 | 149.7 | 150.1 | 150.8 | 150.8 | 151.3 | 150.4 | 150.5 | 148.6 | 150.1 | 145.2 | 147.5 |
| C-6 | 165.3 | 165.3 | 165.3 | 165.5 | 124.7 | 157.4 | 157.3 | 161.6 | 157.4 | 165.2 | 0.23 | 165.2 | 165.2 | 165.1 | 157.7 | 157.7 | 161.2 | 165.3 | 165.5 | 165.2 | 165.1 | 163.6 | 163.2 |
| C-7 | 165.9 | 166.1 | 166.1 | 166.1 | 162.2 | 125.0 | 124.2 | 121.6 | 124.0 | 166.2 | 0.22 | 166.2 | 166.2 | 166.2 | 124.8 | 123.9 | 121.6 | 166.1 | 166.1 | 165.9 | 165.9 | 160.2 | 160.3 |
| C-8 | 156.6 | 156.7 | 156.7 | 156.7 | 157.5 | 152.9 | 152.9 | 154.7 | 152.8 | 156.4 | 0.43 | 156.4 | 157.9 | 156.5 | 152.9 | 152.9 | 154.8 | 156.7 | 156.6 | 156.6 | 156.6 | 156.8 | 156.9 |
| C-9 | 152.0 | 152.2 | 152.1 | 152.2 | 152.6 | 159.5 | 159.5 | 158.0 | 159.5 | 158.9 | 0.44 | 158.9 | 156.4 | 158.9 | 166.1 | 166.1 | 163.2 | 152.1 | 152.0 | 152.1 | 152.0 | 137.5 | 138.0 |
| C-10 | 157.2 | 158.0 | 158.0 | 158.0 | 156.7 | 157.3 | 157.3 | 157.0 | 157.3 | 158.3 | 1.92 | 158.3 | 158.3 | 158.3 | 157.7 | 157.7 | 157.7 | 157.9 | 157.8 | 157.1 | 157.8 | 156.1 | 156.9 |
| C-11 | 171.7 | 171.7 | 171.7 | 171.7 | 171.8 | 171.7 | 171.7 | 171.7 | 171.9 | 163.8 | 0.25 | 163.8 | 165.5 | 163.8 | 164.3 | 164.3 | 165.7 | 171.6 | 171.6 | 171.7 | 171.5 | 171.5 | 171.4 |
| C-12 | 152.2 | 152.3 | 152.3 | 152.3 | 152.6 | 152.9 | 152.9 | 152.9 | 152.9 | 120.0 | 0.46 | 119.5 | 117.0 | 119.7 | 120.3 | 119.4 | 117.2 | 153.3 | 153.2 | 152.2 | 152.1 | 152.1 | 152.3 |
| C-13 | 149.8 | 149.8 | 149.8 | 149.8 | 149.7 | 149.8 | 149.8 | 149.8 | 149.8 | 146.0 | 2.08 | 146.0 | 148.1 | 145.9 | 146.0 | 146.0 | 147.1 | 148.4 | 148.2 | 149.8 | 149.7 | 149.8 | 149.8 |
| C-14 | 135.9 | 136.1 | 136.0 | 136.1 | 136.5 | 141.9 | 141.9 | 141.6 | 141.9 | 144.4 | 0.46 | 144.4 | 142.7 | 144.4 | 150.8 | 150.8 | 148.9 | 135.9 | 135.7 | 135.8 | 135.8 | 135.7 | 135.9 |
| C-15 | 168.4 | 168.4 | 168.4 | 168.4 | 168.3 | 168.8 | 168.8 | 168.9 | 168.8 | 168.6 | 0.28 | 168.6 | 169.0 | 168.6 | 169.3 | 169.3 | 169.3 | 169.6 | 169.2 | 168.3 | 168.1 | 168.2 | 168.2 |
| C-16 | 164.4 | 164.4 | 164.4 | 164.4 | 164.4 | 164.3 | 164.3 | 164.5 | 164.3 | 166.3 | 0.22 | 166.3 | 166.3 | 166.3 | 164.8 | 164.8 | 164.8 | 168.1 | 168.0 | 164.2 | 164.1 | 164.1 | 164.1 |
| C-17 | 136.4 | 136.3 | 136.5 | 136.3 | 136.5 | 136.5 | 136.5 | 136.5 | 136.3 | 145.4 | 0.43 | 145.4 | 144.4 | 145.2 | 145.4 | 145.4 | 144.4 | 128.8 | 128.5 | 136.0 | 135.8 | 136.0 | 135.9 |
| C-18 | 180.7 | 181.0 | 181.8 | 181.1 | 180.7 | 180.9 | 180.7 | 180.7 | 180.9 | 179.9 | 1.12 | 179.9 | 180.0 | 179.9 | 180.0 | 180.0 | 180.0 | 179.4 | 179.5 | 180.7 | 180.8 | 180.2 | 180.2 |
| C-19 | 168.6 | 169.6 | 169.4 | 169.8 | 169.2 | 169.8 | 169.6 | 169.8 | 169.8 | 169.4 | 0.91 | 169.4 | 169.3 | 169.4 | 170.0 | 170.0 | 169.7 | 169.4 | 169.5 | 168.4 | 169.1 | 180.3 | 180.4 |
| C-20 | 157.2 | 157.2 | 157.2 | 157.2 | 157.0 | 157.0 | 157.0 | 157.1 | 156.9 | 157.1 | 0.42 | 157.1 | 157.1 | 156.9 | 157.1 | 157.1 | 157.2 | −16.9 | −17.0 | 156.7 | 156.6 | 156.5 | 156.6 |
| C-21 | 174.5 | 174.7 | 174.5 | 174.8 | 174.5 | 174.5 | 174.4 | 175.5 | 174.2 | 175.3 | 0.88 | 175.3 | 175.3 | 174.8 | 175.2 | 175.2 | 174.3 | 161.3 | 161.0 | 174.0 | 173.8 | 173.7 | 173.8 |
| C-21 | 161.6 | 161.6 | 161.5 | 161.5 | 161.5 | 161.6 | 161.5 | 161.6 | 160.5 | 161.5 | 0.32 | 161.4 | 161.4 | 160.7 | 161.5 | 161.5 | 161.4 | | | 156.3 | 156.2 | 156.1 | 156.2 |
| C-23 | 161.7 | 161.7 | 161.8 | 161.7 | 161.7 | 161.7 | 161.8 | 161.8 | 163.3 | 161.6 | 0.33 | 161.6 | 161.6 | 163.3 | 161.6 | 161.6 | 161.6 | | | 168.7 | 168.3 | 168.4 | 168.5 |
| C-24 | 17.8 | 18.3 | 17.7 | 17.6 | 17.7 | 18.2 | 17.6 | 17.5 | 129.9 | 15.6 | | 17.6 | 17.6 | 129.0 | 18.3 | 17.5 | 17.4 | | | 153.0 | 152.9 | 152.8 | 152.8 |
| C-25 | | | | | | | | | | | | | | | | | | | | 164.5 | 164.4 | 164.3 | 164.3 |
| C-26 | | | | | | | | | | | | | | | | | | | | 170.0 | 169.7 | 169.7 | 169.7 |
| C-27 | | | | | | | | | | | | | | | | | | | | 170.2 | 169.9 | 169.0 | 169.9 |
| $OCH_3$ | 141.5 | | 141.4 | 141.2 | 141.4 | | 141.0 | 141.0 | | | | 141.0 | 141.0 | | | 141.0 | 141.0 | | | | | | |
| $OCH_3$ | | | | 23.4 | | | | 23.6 | | | | | 27.8 | | | | 23.5 | | 21.8 | | | | |
| $COCH_3$ | | | | 171.8 | | | | 171.9 | | | | | 171.9 | | | | 172.0 | | 171.0 | | | | |

**Table II.** Differences between Chemical Shifts (ppm) of Corresponding Carbons of Steroid and Cyclohexane Derivatives

| | $\delta_n$(coprostane) − $\delta_n$(cholestane) | $\delta_n$(epicoprostanol) − $\delta_n$(cholestanol) | $\delta_n$(coprostane) − $\delta_n$(epicoprostanol) | $\delta_n$(cholestane) − $\delta_n$(cholestanol) | $\delta_{n'}$(A) − $\delta_{n'}$(B)[a] |
|---|---|---|---|---|---|
| C-1 | +1.3 | +1.6 | −2.1 | −1.8 | −2.5 |
| C-2 | +1.0 | +0.8 | +9.4 | +9.2 | +7.9 |
| C-3 | −0.2 | −0.6 | +44.7 | +44.3 | +43.2 |
| C-4 | +1.3 | +1.7 | +9.1 | +9.5 | +7.9 |
| C-5 | +3.4 | +2.6 | −1.5 | −2.3 | −2.5 |
| C-6 | +1.6 | +1.9 | +0.1 | +0.4 | |
| C-7 | +5.7 | +5.6 | 0.0 | −0.1 | |
| C-8 | −0.2 | −0.3 | 0.0 | −0.1 | |
| C-9 | +14.6 | +14.0 | +0.1 | −0.5 | |
| C-10 | +1.0 | +0.9 | −0.7 | −0.8 | −1.6 |
| C-19 | −11.9 | −10.3 | −0.7 | −0.1 | |

[a] The 4 position of *tert*-butylcyclohexane (A) and the 1 position of *trans*-4-*tert*-butylcyclohexanol (B) were taken to correspond to C-3 of the steroids.

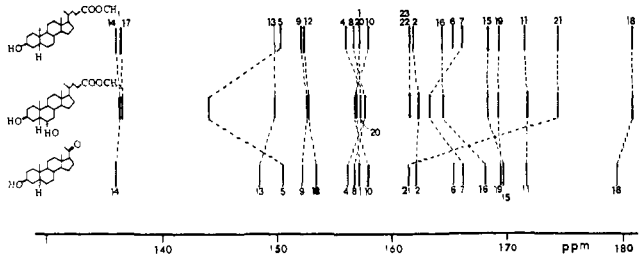



Figure 9. Correlation diagram of $^{13}C$ shifts of **1a**, **2a**, and 5β-pregnan-3α-ol-20-one (**7**).

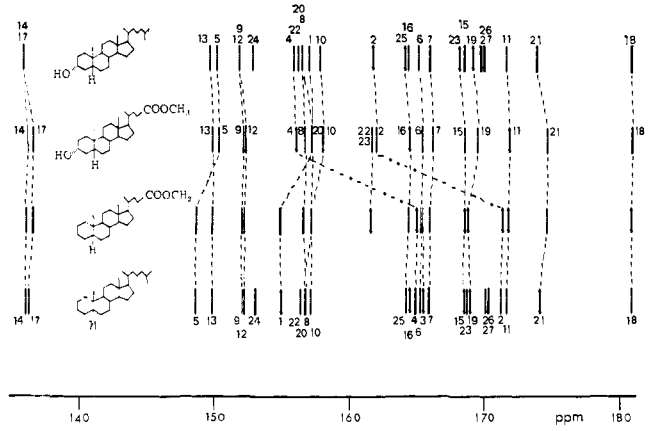



Figure 10. Correlation diagram of the $^{13}C$ shifts of epicoprostanol (**10**), methyl lithocholate (**1a**), methyl cholanate (**8a**), and coprostane (**9**).

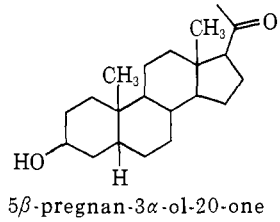
shift of the C-11 resonance of 7.9 and 7.2 ppm, respectively.[12] The lanthanide shift for C-11 with **2a** and **4a** is larger than for the $CH_2$ resonances in the D ring (see Figures 6 and 7) and, finally, acetylation of a C-12 hydroxyl shifts the C-11 resonance upfield 1.7 ppm for **2**, and 1.4 ppm for **4**.

There are carbon resonances of **1** and **3** which are comparable in position to those assigned to C-12 for trans steroids.[2] However, from the work of Eggert and Djerassi,[14] it is clear that previous assignments[2] for C-12 and C-16 should be reversed. This reversal fits well with the data obtained for **2** and **4** for which the resonances at about 153 ppm are absent. The C-12 resonances disappear from their normal location in **2** and **4**. The peak corresponding to the quaternary C-13 can be distinguished from C-10 as described earlier. The shift of C-14 in **1** is as in the trans steroids. This reasonance moves to higher field by 6.5 ppm in **3**, 8.3 ppm in **2**, and a total of 14.1 ppm in **4** because of 1,3-diaxial interactions. The acetate shifts of this carbon are −0.3 ppm for **3** and −1.4 ppm for **2**, which agree with the total of −1.9 ppm observed for acetylation of **4** (see Table I and Figures 5–7).

**The D-ring assignments** were straightforward. Thus, C-15 gives a methylene carbon resonance which shows a small lanthanide shift only when there is a hydroxyl at C-7. The shift of this carbon is the same as in trans steroids. The resonances assigned to C-16 are in a rather constant position for all of the compounds studied here (except **7**) and do not show lanthanide or acetylation shifts.[14] They have appropriate relaxation times and sfor patterns for $CH_2$ groups. The C-17 keto group of **7** moved the C-16 resonance upfield by 4 ppm, but affected the resonance of the most distant C-12 by only 1 ppm.

The resonance of C-17 of **1** and **3** is located as in the trans steroids and is shifted by about 9 ppm upfield by substitution of the axial hydroxyl at C-12, as in **2** and **4**. This shift is somewhat larger than normal for the corresponding axial interaction with a cyclohexane[12] and may reflect the general tightness of a trans-fused cyclopentane ring with cis vicinal substituent groups.

**Angular methyl group and side-chain carbon resonances** were allocated to specific positions as follows. First, C-19 is easily differentiated from C-18 because it comes at about 10 ppm to lower field. This is expected because with a cis A/B ring junction, the four axial interactions experienced by the C-19 methyl in trans steroids with the hydrogens on C-2, C-4, C-8, and C-11 are reduced twofold, as will be seen by comparing **6a** and **6b**. The resonance of C-21 can be differentiated from those of C-18 and C-19 by its sfor pattern, which is broadened by residual coupling with the hydrogen on C-20. All three methyl carbons have larger $T_1$ relaxation times than all of the other hydrogen-bearing carbons, because internal rotation reduces their correlation times relative to the others.[10] The peak for C-20 is the only invariant CH carbon resonance of compounds **1–5** and shows no lanthanide shift. Its assignment is confirmed by the spectrum (Figure 9) of 5β-pregnan-3α-ol-20-one (**7**), which also serves to differentiate the resonances of C-12, C-15, and C-16 from those of C-22 and C-23 as well as C-17 from the other CH carbon resonances, and C-21 from the other methyl resonances. The signals from C-22 and C-23




5β-pregnan-3α-ol-20-one

**7**

methylene carbons have constant positions in the acids (**1–5**) and methyl esters (**1a–5a**). They do not give lanthanide shifts for the esters and can be distinguished from one another by reduction to the corresponding alcohols **2b–3b**, which moves the C-23 signal 1.5 ppm to higher field and that of C-22 to somewhat lower field, as has been found for similar reductions of carboxylic groups.[15] The $T_1$ relaxation times for C-22 and C-23 are a bit larger than for the other $CH_2$ carbons which suggests a shorter correlation time for these carbons.

**Cholanic acid (8)** was examined in the form of its methyl ester **(8a).** As will be seen in Figure 10, C-2 and C-4 are upfield by 8.8 and 9.1 ppm with respect to **1**, which corresponds to changes of 9.0 and 10.5 ppm for the cholestane/cholestanol pair (**11** and **12**) with the A/B ring junction trans (see Table I). The signals from

(14) H. Eggert and C. Djerassi, *J. Amer. Chem. Soc.*, **95**, 3710 (1973); we concur that our earlier assignments[2] were incorrect and we appreciate the information provided by Professor Djerassi, in advance of publication, which avoided awkward assignments for C-12 and C-16 for **2** and **4**. The C-12 and C-16 line positions for **7–12** given in Table I reflect the new C-12 and C-16 assignments.

(15) J. B. Grutzner, M. Jautelat, J. B., Dence, R. A. Smith, and J. D. Roberts, *J. Amer. Chem. Soc.*, **92**, 7107 (1970).

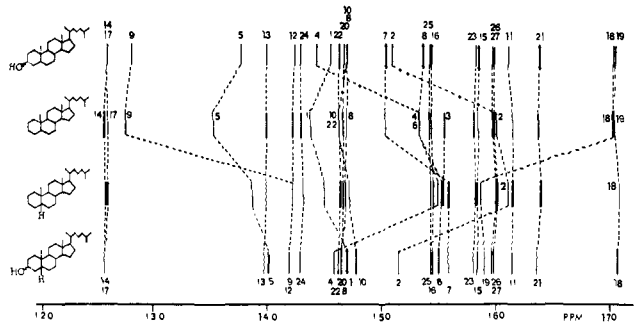
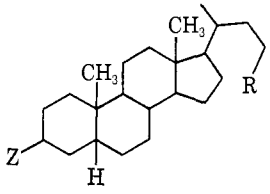
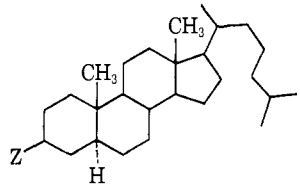


Figure 11. Correlation diagram of the $^{13}C$ shifts of cholestanol (**12**), cholestane (**11**), coprostane (**9**), and epicoprostanol (**10**).



**8**, cholanic acid (Z = H, R = $CO_2H$);
**8a**, methyl cholanate (R = $CO_2CH_3$)
**9**, coprostane (Z = H, R = $CH_2CH(CH_3)_2$)
**10**, epicoprostanol (Z = $\alpha$-OH, R = $CH_2CH(CH_3)_2$)



**11**, cholestane (Z = H)
**12**, cholestanol (Z = $\beta$-OH)

C-1 and C-5 of **8a** are 2.3 and 2.6 ppm downfield relative to the respective signals of **1**. The corresponding shifts for the change from **12** to **11** are 2.0 and 2.3 ppm. Removal of the equatorial hydroxyl shifts the C-3 resonance of both **1** and **12** about 44 ppm, which fits well with the 43.2-ppm change resulting from the removal of the equatorial hydroxyl from *trans*-4-*tert*-butylcyclohexanol.[12] Small shift changes are also observed for C-9 and C-10 between **1** and **8a**; the rest of the resonances are in essentially identical positions.

To further characterize the differences associated with steroids having cis and trans A/B ring junctions, we have determined the shifts of coprostane (**9**) and epicoprostanol (**10**). The side chain carbon peaks can be assigned by comparing **10** with **1a** (see Figure 10). With this knowledge, the signals of **9** can be allocated by comparison with **8a**.

The cis and trans steroids differ most significantly at C-9 and C-19 (see Figure 11) and these signals will surely be of prime value to characterize the nature of the ring junction. Further aid to distinction comes from the substantial upfield shifts of C-5 and C-7 in the cis steroids. It will be seen that the pattern of differences obtained from $\delta_n$(coprostane) − $\delta_n$(cholestane) is wholly comparable to that for $\delta_n$(epicoprostanol) − $\delta_n$(cholestanol). Furthermore, $\delta_n$(coprostane) − $\delta_n$(epicoprostanol) and $\delta_n$(cholestane) − $\delta_n$(cholestanol) are very comparable to the appropriately corresponding $\delta_{n'}$(*tert*-butylcyclohexane) − $\delta_{n'}$(*trans*-4-*tert*-butylcyclohexanol) (see Table II). This

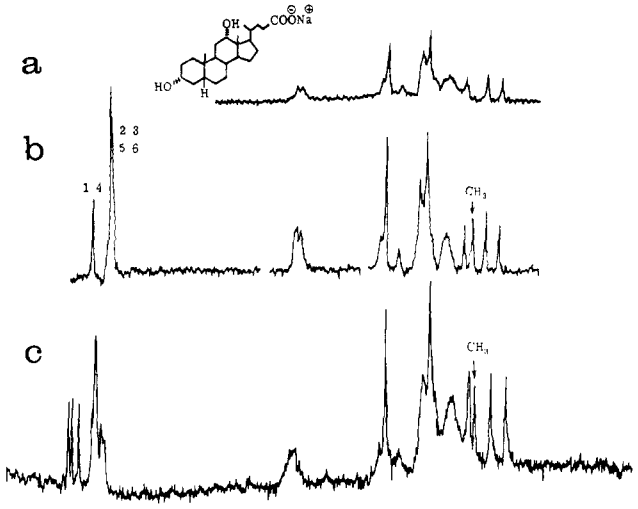


Figure 12. Carbon-13 nmr spectrum at 15.09 MHz of sodium desoxycholate in: (a) water solution; (b) with 2 mol equiv of *p*-xylene; and (c) with 2 mol equiv of 2-methylnaphthalene.

means that the effect of substitution on an equatorial hydroxyl group is very similar in all of these chair-type cyclohexane rings.

**Some Choleic Acids (Inclusion Compounds of Desoxycholic Acid, 2).** An early controversy about the natures of two bile acids with one less oxygen than cholic acid (**4**), desoxycholic acid (**2**),[16] and choleic acid[17] was finally resolved by the finding[18] that, while **2** was a single substance, "choleic acid" was an inclusion compound of **2** and fatty acids. Formation of such materials with **2** is not unique to fatty acids. Molecules of desoxycholic acid associate with one another to form channels which can include a variety of guest molecules. Depending upon the molecular sizes of the guest substances, the combinations may crystallize in constant stoichiometric ratios.[19] The association clearly persists in solution, as can be seen from the way in which aqueous solutions of the sodium salt of **2** take up a variety of water-insoluble substances.[20] The cmr spectrum of the sodium salt of **2** in $D_2O$ is very much affected by the association (Figure 12a) (all of the resonances of those carbons carrying directly bonded protons, except those of the angular methyl groups, being substantially broadened as the result of slowing of molecular tumbling and an increase in dipolar-induced relaxation). The angular methyl resonances are not affected to the same degree because of rapid rotation around their C–C bonds. The quaternary carbons, C-10, C-13, and C-24, without directly attached protons, undergo less efficient dipolar relaxation and their resonances also remain rather sharp.

If one saturates an aqueous solution of the sodium salt of **2** with *p*-xylene, the guest molecules are taken up in a molal ratio of about 2:1. The cmr spectrum (Figure 12b) clearly shows the rather narrow resonances of the *p*-xylene carbons, and it is evident that the xylene molecules are not constrained to tumble at the same

(16) F. Mylius, *Chem. Ber.*, **19**, 374 (1886).
(17) P. Latschinoff, *ibid.*, **20**, 1043 (1887).
(18) H. Wieland and G. Sorge, *Hoppe-Seyler's Z. Physiol. Chem.*, **97**, 1 (1916).
(19) F. Cramer, "Einschlussverbindungen," Springer-Verlag, West Berlin, 1954.
(20) *Cf.* L. F. Fieser and M. Fieser, "Steroids," Reinhold, New York, N. Y., 1959, pp 60–61.

slow rate as the host aggregates. The larger 2-methylnaphthalene is also taken up in about a 2:1 ratio, and the cmr spectrum (Figure 12c) shows that the resonances of the CH carbons are more broadened relative to those of the quaternary carbons than for *p*-xylene. Indeed, some additional broadening of the carbon resonances of the salt of **2** seems evident from the spectrum. With either *p*-xylene or 2-methylnaphthalene, the methyl resonances of the included materials are sharp. This is expected on the basis that spin rotation is the important relaxation mechanism with rapidly rotating methyl groups as those of toluene[21] and duene.[22] Apparently, the rates of the methyl rotations are not reduced in the included molecules and the reduced rate of tumbling, as of 2-methylnaphthalene, does not affect the relaxation rate of the methyl carbons.

It seems that the cmr spectra of choleic acids formed with properly sized molecules could be useful in assigning resonances to particular carbons as well as providing a rapid method of differentiating between various relaxation mechanisms. The relatively narrow groupings of the resonances of **2** should be helpful in these respects.

**Acknowledgment.** We are pleased to acknowledge the courtesy of Varian Associates in providing the use of a FT-equipped XL-100 spectrometer in their Applications Laboratory for taking some of the early spectra and measurements of relaxation times.

(21) C. F. Schmidt, Jr., and S. I. Chan, *J. Chem. Phys.*, in press.
(22) K. F. Kuhlmann and D. M. Grant, *ibid.*, **55**, 2998 (1971).

# Nybomycin. VII. Preparative Routes to Nybomycin and Deoxynybomycin[1,2]

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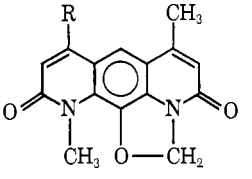
*Contribution from the Department of Chemistry, University of Illinois, Urbana, Illinois 61801.* *Received December 16, 1972*

**Abstract:** Syntheses of the antibiotics nybomycin (**1**) and deoxynybomycin (**2**) from *o*-anisidine, in twelve and eight steps, respectively, are described.

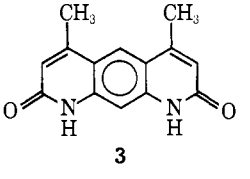
The antibiotic nybomycin was isolated several years ago in two laboratories from streptomycete cultures;[4,5] it was found to be quite active against Gram-positive and some Gram-negative bacteria, but its very limited solubility restricts its *in vivo* activity.

The molecular structure of nybomycin has recently been assigned as **1**.[6] One of the key intermediates in establishing structure **1**, deoxynybomycin (**2**), was initially obtained from nybomycin by hydriodic acid reduction,[7] but it has very recently been reported by Umezawa, *et al.*, as an antibiotic in its own right, being produced by *Streptomyces hyalinum* n. sp. Hamada et Yakayama.[8] The biological activity of deoxynybomycin and the observation that nybomycin acetate and dichloroacetate are more active than the parent compound, especially against staphylococci,[9] lend interest to the development of synthetic methods which might be employed in preparing compounds of this type for investigations of structure–activity relationships. The present paper describes in detail preparative studies culminating in the total synthesis of deoxynybomycin and nybomycin and leading to a number of related heterocyclic compounds.

**Formation of a Model Oxazoline.** The ring system of nybomycin provides the first naturally occurring example of a pyridoquinolone (diazaanthracenedione), and a reduction product (**3**) from deoxynybomycin con-



1, R = $CH_2OH$
**2**, R = $CH_3$



**3**

taining this ring system was synthesized earlier[10] from *m*-xylene. However, the most striking structural feature in the nybomycin ring system is the fused 4-oxazoline nucleus; this heterocyclic system, with a saturated linkage between nitrogen and oxygen, appears not to have been reported before in natural products, although its oxygen analog, the methylenedioxy group, is found in many natural products, including casimiroin,[11] a methylenedioxy-*N*-methylquinolone, and the streptovaricin antibiotics.[12] In contrast to the numerous

(1) This work has been the subject of two preliminary reports: (a) R. M. Forbis and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.*, **92**, 6995 (1970); (b) *J. Antibiot.*, **24**, 326 (1971).
(2) Taken from the Ph.D. Thesis of R. M. Forbis, University of Illinois, February 1971.
(3) National Science Foundation Predoctoral Fellow.
(4) F. Strelitz, H. Flon, and D. N. Asheshov, *Proc. Nat. Acad. Sci. U. S.*, **41**, 620 (1955).
(5) T. E. Eble, G. A. Boyack, C. M. Large, and W. H. DeVries, *Antibiot. Chemother.*, **8**, 627 (1958).
(6) K. L. Rinehart, Jr., G. Leadbetter, R. A. Larson, and R. M. Forbis, *J. Amer. Chem. Soc.*, **92**, 6994 (1970).
(7) K. L. Rinehart, Jr., and H. B. Renfroe, *J. Amer. Chem. Soc.*, **83**, 3729 (1961).
(8) H. Naganawa, T. Wakashiro, A. Yagi, S. Kondo, T. Takida, M. Hamada, K. Maeda, and H. Umezawa, *J. Antibiot.*, **23**, 365 (1970).
(9) T. D. Brock and W. T. Sokolski, *Antibiot. Chemother.*, **8**, 631 (1958).
(10) G. Leadbetter and K. L. Rinehart, Jr., *Can. J. Chem.*, **43**, 1625 (1965).
(11) B. Weinstein and T. A. Hylton, *Tetrahedron*, **20**, 1725 (1964).
(12) A review: K. L. Rinehart, Jr., *Accounts Chem. Res.*, **5**, 57 (1972).