

Differentiation between the 25*R*- and 25*S*-isomers of 5 β -cholestane-3 α ,7 α ,26-triol by ¹³C NMR spectroscopy

A. K. Batta, T. H. Williams, G. Salen, D. N. Greeley, and S. Shefer

Department of Medicine, College of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, NJ 07103; Chemical Research Department, Hoffmann-LaRoche, Inc., Nutley, NJ 07110¹; Veterans Administration Hospital, East Orange, NJ 07019; Cabrini Medical Center, New York, NY 10003; and The Public Health Research Institute of the City of New York, Inc., New York, NY 10016

Summary This study was designed to examine whether ¹³C nuclear magnetic resonance (NMR) spectroscopy can be used to differentiate between the 25*R* and 25*S* dia-

Abbreviations: NMR, nuclear magnetic resonance; Me₄Si, tetramethylsilane; ppm, parts per million; sfor, single-frequency off-resonance; GLC, gas-liquid chromatography.

¹ T. H. Williams and D. N. Greeley.

stereoisomers of 5 β -cholestane-3 α ,7 α ,26-triol, a key intermediate in the biosynthetic pathway of chenodeoxycholic acid. Chemical shift values were assigned to the individual carbon atoms with the help of model compounds and multiplicity in the single-frequency off-resonance decoupled spectra. It was found that the corresponding carbons 1–20 afforded identical chemical shifts for both compounds, whereas five of the remaining side-chain carbons gave observed shift differences of 0.05–0.20 ppm. Thus ¹³C NMR can be used as an additional tool to distinguish between the two 5 β -cholestanetriols isomeric at C-25.—**Batta, A. K., T. H. Williams, G. Salen, D. N. Greeley, and S. Shefer.** Differentiation between the 25*R*- and 25*S*-isomers of 5 β -cholestane-3 α ,7 α ,26-triol by ¹³C NMR spectroscopy. *J. Lipid Res.* 1980. **21**: 130–135.

Supplementary key words stereospecificity of 26-hydroxylation reactions in bile acid synthesis

The generally accepted mechanism whereby the cholesterol side chain is degraded to form bile acids involves 26-hydroxylated intermediates (1, 2). The hydroxylation at C-26 is accompanied by the introduction of asymmetry at C-25 and thus, two diastereoisomers, 25*R* and 25*S* of the corresponding bile

alcohols are possible. Recent studies in our laboratory on the biosynthetic pathway of chenodeoxycholic acid (3, 4) have demonstrated that the microsomal and mitochondrial 26-hydroxylase systems in rat and human liver follow a stereochemical course during the formation of 5 β -cholestane-3 α ,7 α ,26-triol (25*R* and 25*S*). The two triols have been synthesized in the laboratory (5) and show identical GLC and mass spectral characteristics. They can only be separated by multiple development thin-layer chromatography (5) and the configuration at C-25 has been assigned by comparison of their molecular rotation differences with those of (25*R* and 25*S*)-5 β -cholestane-3 α ,7 α ,12 α ,26-tetrols (6). Lida et al. (7) have described a proton magnetic resonance method for assignment of configuration to epimeric secondary alcohols in sterols but the validity of their method in the case of primary alcohols has yet to be established. Recently, ¹³C NMR spectroscopy has been employed to assign

the pro-*R* and pro-*S* configuration to the terminal methyl groups in cholesterol side chain (8, 9). The present report describes the differentiation between (25*R*) and (25*S*) 5 β -cholestane-3 α ,7 α ,26-triols by this technique.

Materials and methods

Preparation of compounds: (25*R*)- and (25*S*)-5 β -cholestane-3 α ,7 α ,26-triols were synthesized according to Dayal et al. (5). (25*R*)-5 β -cholestane-3 α ,7 α ,26-triol had a melting point of 155–157°C and $[\alpha]_D^{25} = +15.9^\circ$, and (25*S*)-5 β -cholestane-3 α ,7 α ,26-triol had a melting point of 138–140°C and $[\alpha]_D^{25} = +8.8^\circ$. 2-Methylpentanol was obtained from Union Carbide Chemical Co., Hackensack, NJ.

¹³C NMR spectra: The isomeric bile alcohols (10 mg) were each dissolved in 0.35 ml CDCl₃ containing 1% tetramethylsilane (Me₄Si) (v/v) as an internal reference. The ¹³C NMR spectra were recorded at 25.2

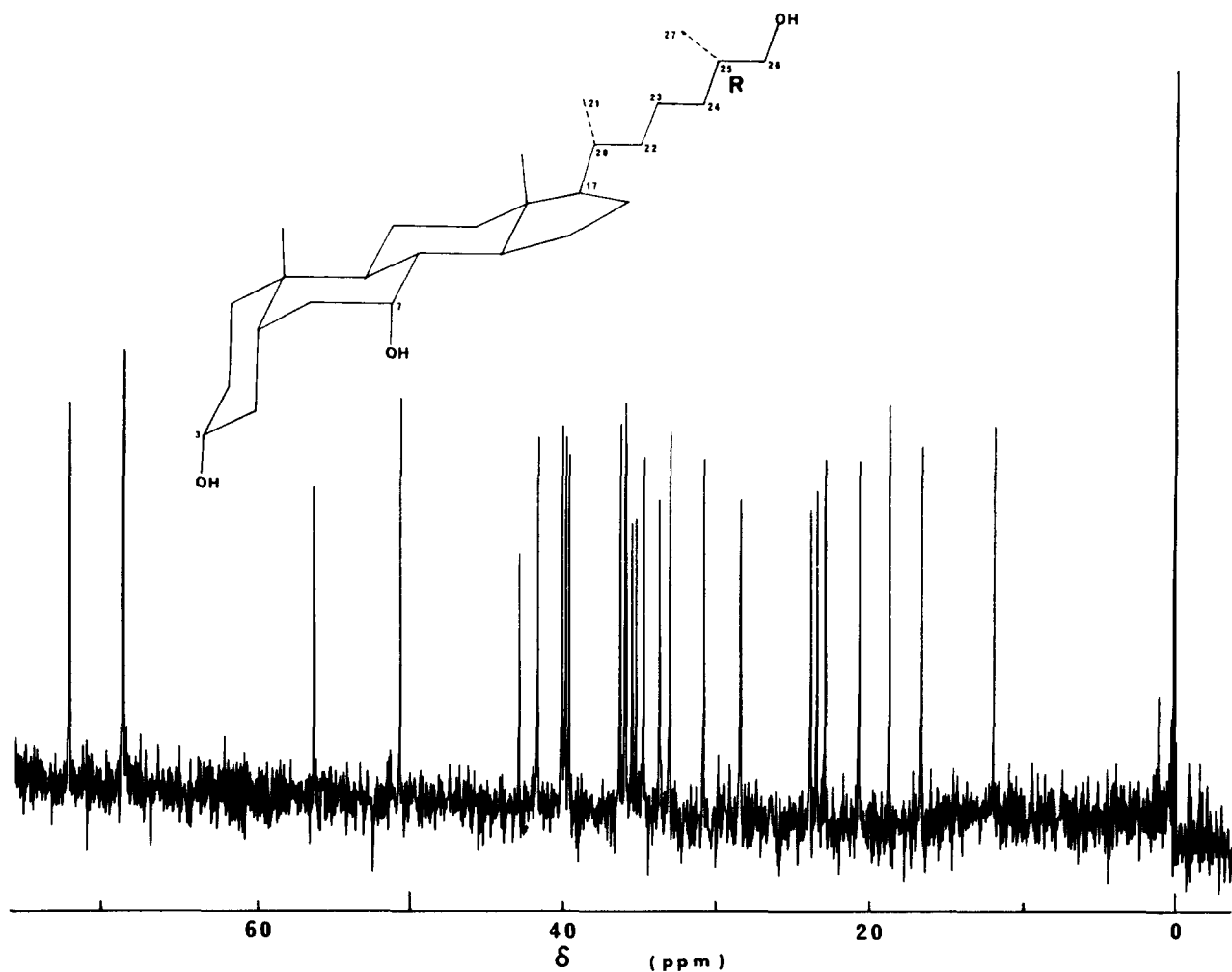


Fig. 1. Proton noise-decoupled ¹³C NMR spectrum of (25*R*) 5 β -cholestane-3 α ,7 α ,26-triol. Assignment of chemical shifts to individual carbons is shown in Table 1.

TABLE 1. ^{13}C NMR chemical shifts^a for (25R) and (25S) 5 β -cholestane-3 α ,7 α ,26-triols

Carbon	δ Values	Carbon	δ Values	Carbon	δ Values	
					(25R)	(25S)
1	35.34 (T) ^b	11	20.60 (T)	20	35.74 (D)	35.74 (D)
2	30.62 (T)	12	39.71 (T) (39.64, 39.80) ^c	21	18.66 (Q)	18.71 (Q)
3	71.82 (D) ^b	13	42.57 (S)	22	36.11 (T)	36.21 (T)
4	39.71 (T) (39.64, 39.80) ^c	14	50.41 (D)	23	23.33 (T)	23.33 (T)
5	41.51 (D)	15	23.67 (T)	24	33.55 (T)	33.68 (T)
6	34.59 (T)	16	28.30 (T)	25	35.74 (D)	35.74 (D)
7	68.40 (D)	17	56.07 (D)	26	68.26 (T)	68.10 (T)
8	39.34 (D)	18	11.76 (Q) ^b	27	16.53 (Q)	16.73 (Q)
9	32.84 (D)	19	22.80 (Q)			
10	35.01 (S) ^b					

^a Chemical shifts were measured in ppm relative to tetramethylsilane.

^b The multiplicity of peaks in the spectra is expressed as S, singlet; D, doublet; T, triplet; Q, quartet.

^c Values obtained for (25R) 5 β -cholestane-3 α ,7 α ,26-triol. Individual assignment to C-4 and C-12 could not be made in this case.

MHz using a Fourier-transform-equipped Varian XL-100 spectrometer. All samples were analyzed in tubes (5 mm o.d.) at probe ambient temperatures of 27°C. The number of spectral accumulations were 58–60 K and the interferograms were accumulated

with 8 K of memory. Sweep widths of 6000 Hz or 5500 Hz were used, corresponding to 1.33 Hz per address (0.06 ppm) or 1.45 Hz per address (0.05 ppm). Chemical shifts are expressed in parts per million (ppm) relative to Me₄Si as internal standard. The ^{13}C NMR

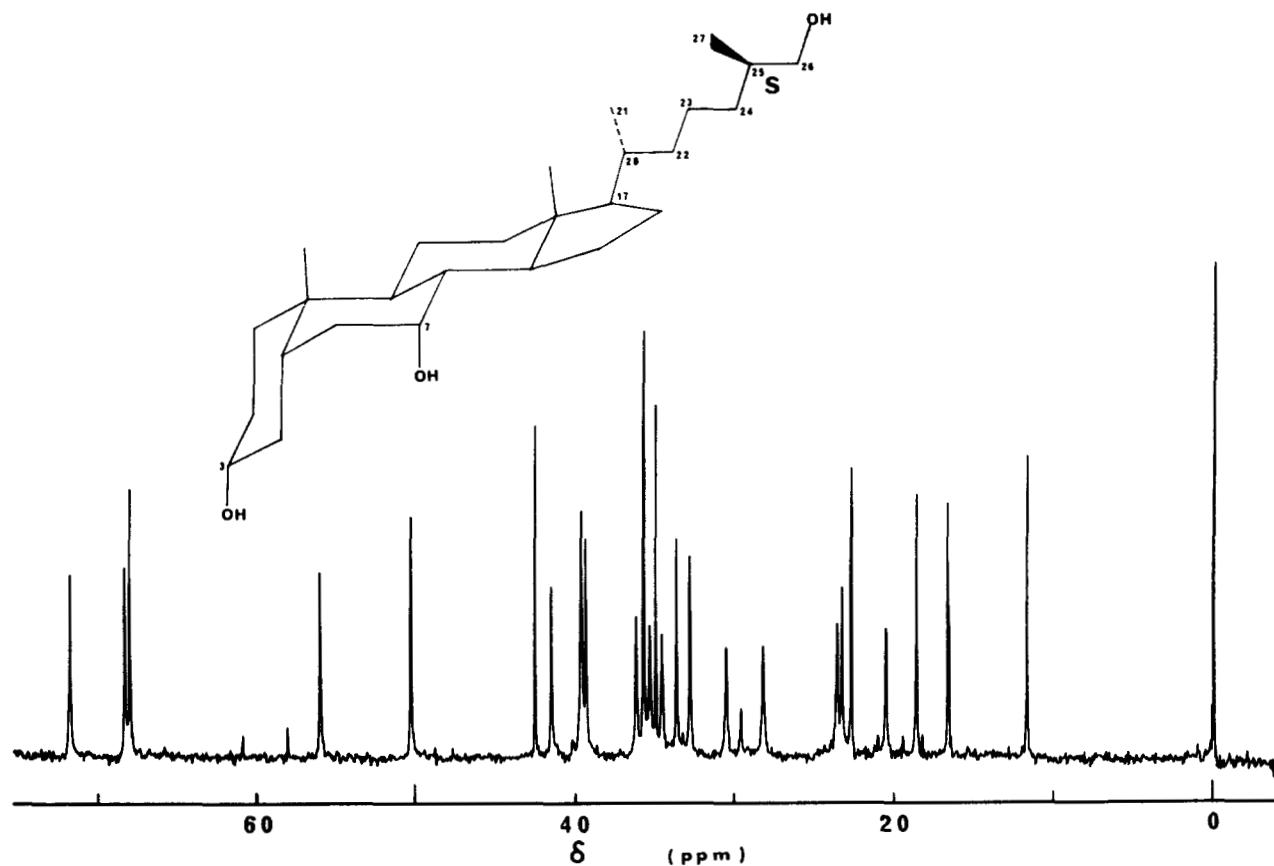


Fig. 2. Proton noise-decoupled ^{13}C NMR spectrum of (25S) 5 β -cholestane-3 α ,7 α ,26-triol. Assignment of chemical shifts to individual carbons is shown in Table I. Peaks of small magnitude at 829.68, 58.06, and 60.86 ppm are due to impurities.

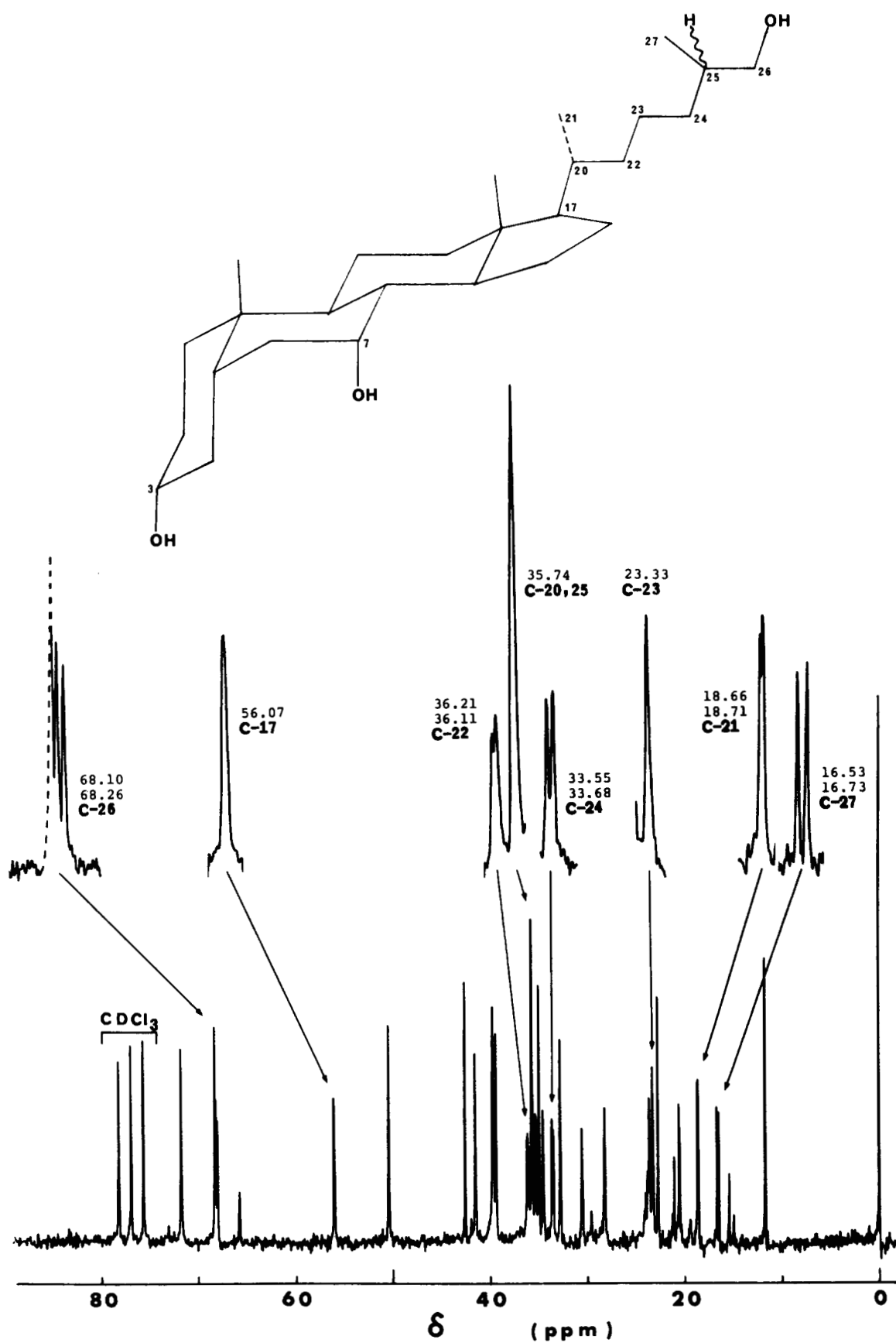


Fig. 3. Proton noise-decoupled ^{13}C NMR spectrum of a mixture of the (25R) and (25S) isomers of 5 β -cholestane-3 α ,7 α ,26-triol. Peaks due to carbon atoms in the side-chain (including C-17) are also shown in expanded plots. Peaks of small magnitude at δ 15.46, 21.12, 29.66, and 65.81 are due to impurities.

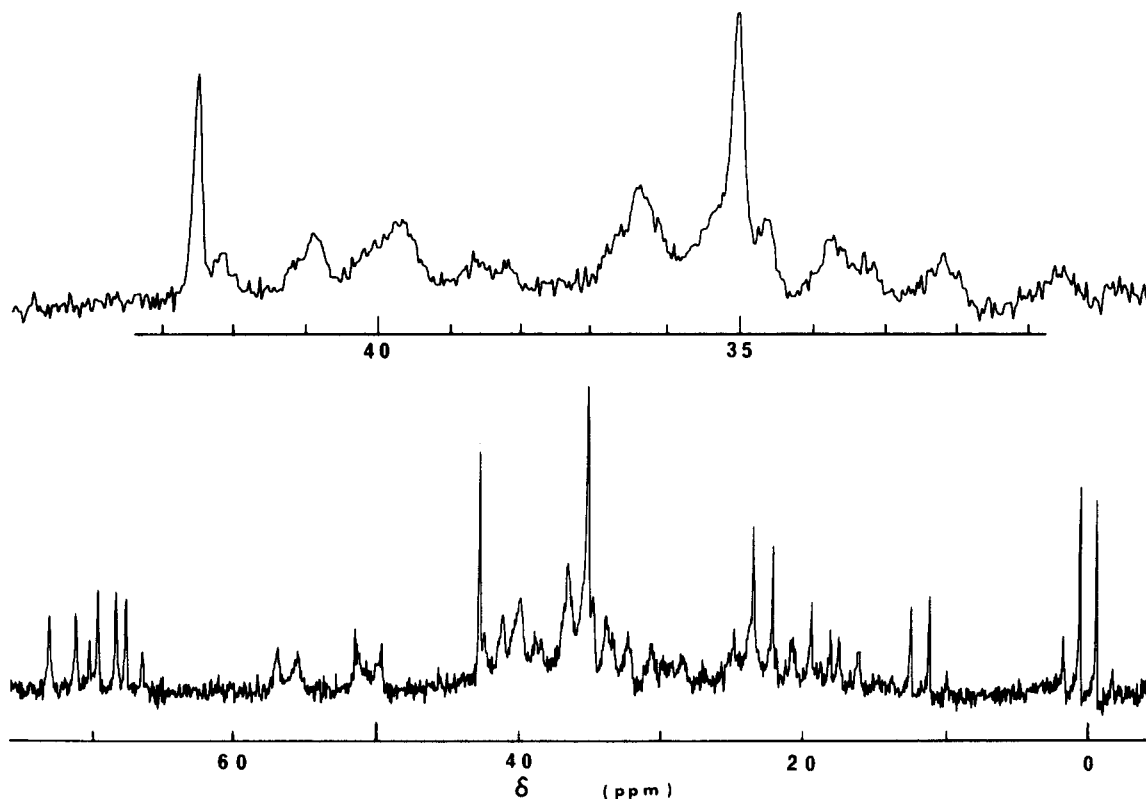


Fig. 4. sfor ^{13}C NMR spectrum of (25*S*) 5 β -cholestane-3 α ,7 α ,26-triol. The sfor spectrum between δ 31–43 ppm is also shown in an expanded plot.

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-resonance (sfor) proton decoupling mode.

Results and discussion

The chemical shift data for the two 5 β -cholestane-3 α ,7 α ,26-triols and for a mixture of the two triols are given in **Table 1** and their ^{13}C NMR spectra are shown in **Figs. 1, 2** and **3** respectively. The substituents

attached to the various carbon atoms could be determined from a detailed study of the sfor spectra and the sfor ^{13}C NMR spectrum of (25*S*) 5 β -cholestane-3 α ,7 α ,26-triol is shown in **Fig. 4**. The observed multiplicity of the peaks facilitated the assignment of chemical shifts to the various carbon atoms and has been incorporated in **Table 1**, along with the chemical shifts. As seen from this table, the resonances due to all the 27 carbons were resolved, except for two pairs (carbons 4 and 12 and carbons 20 and 25) in case of (25*S*) 5 β -cholestane-3 α ,7 α ,26-triol and one pair (carbons 20 and 25) in case of (25*R*) 5 β -cholestane-3 α ,7 α ,26-triol. Resonances due to carbons 4 and 12 were identical when the 25*S* and the 25*R* isomers were mixed together.

Using methyl 3 α ,7 α -dihydroxy-5 β -cholan-24-oate as a model compound (10), the carbons C-1 to C-20 could be systematically assigned, based on the multiplicity of the signals in the sfor spectra and comparison of chemical shifts. 2-Methylpentanol served as a model (**Fig. 5**) for the assignment of chemical shift values to carbons C-24 to C-27 in the side chain. A γ -effect of C-20 (-2.69 ppm) and δ -effect of C-17 and C-21 ($+0.25$ ppm each) (11) were taken into account for assignment of C-24. Carbons 21, 22 and 23 gave chemical shifts similar to those observed for the

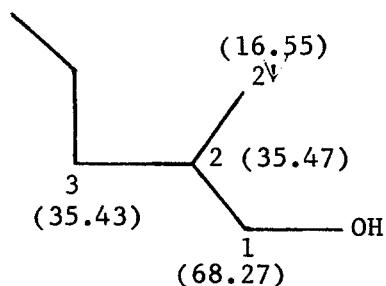



Fig. 5. Observed ^{13}C NMR chemical shifts for C-1, C-2, C-2' and C-3 in 2-methylpentanol.

cholesterol side chain (12, 13), carbon 23 was upfield by 0.6 ppm due to the δ -effect of the -OH group at C-26.

As expected, the two compounds showed identical chemical shifts for carbons C-1 to C-20, being far away from the chiral carbon C-25, and shift differences were observed only for carbons in the side chain as pointed out in Fig. 3. Identity in chemical shifts was found for the chiral carbons C-20 and C-25, but not for the adjacent carbons, except for C-17, for which the shift difference was too small to be resolved. Thus, the oxygenated carbon C-26 showed a downfield shift (+0.16 ppm) for the 25*R* compared to the 25*S* isomer, whereas the non-oxygenated carbons C-21, C-22, C-24, and C-27 exhibited upfield shifts (-0.05, -0.10, -0.13, and -0.20 ppm, respectively). Carbon-23, furthest from both asymmetric carbons (C-20 and C-25) in the side chain, was isochronous in the spectra of the two compounds.

¹³C NMR spectroscopy can thus be used to study the stereospecificity of microsomal and mitochondrial 26-hydroxylation in bile acid biosynthesis. However, it may sometimes be necessary to use precursors enriched in ¹³C since it is difficult to isolate milligram quantities from biological tissues. 

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