Carbon-13 Nuclear Magnetic Resonance Spectral Analysis of 16-Membered Macrolide Antibiotics[1]

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Abstract: The natural abundance ^{13}C NMR spectra of the macrolide antibiotics Leucomycin-A_3 and A_5, Spiramycin III, Magnamycin-A and B, Chalcomycin, Neutramycin, Tylosin, and Cirramycin-A_1 were recorded. Chemical shifts were assigned with the help of the spectra of both anomeric forms of the model sugar components mycarose, mycaminose, calcose, mycinose, and forosamine. The hitherto unknown anomeric configuration of forosamine of the Spiramycins as well as the location of the disaccharide moiety in Tylosin were determined.

Since the advent of nuclear magnetic resonance spectroscopy in the 1950's, a vast amount of research has been directed toward the structure elucidation and conformational analysis of complex antibiotics.[3] Application of NMR spectroscopy has been focused mainly on the hydrogen nucleus. However, recent advances in the ^{13}C magnetic resonance area have increased greatly the scope and utility of NMR spectroscopy in structural studies of high molecular weight substances.

The importance of the 16-membered macrolides produced by various Actinomycetes as pharmaceuticals has become increasingly greater with the recent discovery of numerous related substances[4] in addition to the commercially available Carbomycin,[5,8d] Leucomycin,[6] Tylosin,[7] and Spiramycin.[8a-c,e]

The elucidation of the structure of these compounds brought out some interesting features presenting challenging biogenetic problems; some of them have already been explored;[9] others are under study in various laboratories using ^{13}C labeled precursors.[10] Although the constitution of most of the compounds studied in this paper has been well established, the structure of the Spiramycins and that of Tylosin **23** was only incompletely known.

This paper describes the results of the first systematic investigation of the ^{13}C NMR spectra of 16-membered macrolide antibiotics which was undertaken in order to lay the groundwork for structure analysis and biosynthetic studies using this new diagnostically powerful research tool.

The proton-decoupled natural abundance ^{13}C FT NMR spectra at 22.63 MHz of the various macrolides have been examined in 0.04–0.3 *M* $CDCl_3$ solution.[11] These antibiotics present at first sight an especially difficult problem from the point of view of the ^{13}C signal assignments since many of their resonances fell within a fairly narrow shift range. Single-frequency off-resonance decoupled (SFORD) spectra were helpful only in the analysis of the high field part of the spectra. In the critical 65–80 ppm region, all carbon atoms exhibited doublet multiplicities except the C-3″ mycarose signal and C-24 in case of Tylosin **23,** Chalcomycin[12] **25,** and Neutramycin[13] **26.**[14] In order to assign the resonances of these compounds to specific carbons, an analysis of the ^{13}C NMR spectra of their individual sugar components became a necessity (Table I).

Results and Discussion

Spectral Analysis of the Sugar Components of the Antibiotics. The 16-membered macrolide antibiotics each contain two or three hexoses; Cirramycin-A_1[15] **24** is the only exception in which the lactone ring is attached to only one sugar. The hexoses are generally of five types:[18] L-mycarose, D-mycaminose, D-chalcose, D-mycinose, and D-forosamine (see Figure 1). By acid hydrolysis of Leucomycin-A_3[6] **17** and Spiramycin III[8a-c] **20,** mycarose, 4-*O*-isovaleryl mycarose, mycaminose, and forosamine have been isolated by published procedures.[16] Chalcose and mycinose have been synthesized using slight modifications of known methods.[17]

The ^{13}C NMR spectra of these five sugars have been examined either as their methyl glycosides or as free hexoses, some of them as pure samples, others as anomeric mixtures. In the latter case, one component of the mixture predominated except in case of the methyl mycaminosides **5** and **6.** The resonance intensity differences between the signals characterizing the carbons of each component allowed unambiguous assignments to be made in the specific compounds. In case of the methyl mycaminosides **5** and **6** the shifts fell in an especially narrow range; moreover, as a consequence of the nearly equal quantities present in the anomeric mixture (vide supra), specific assignments are not unequivocal.

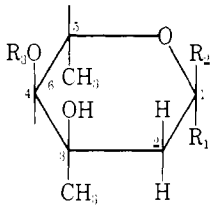
The 99.4 and 98.5 ppm signals of β- and α-methyl mycarosides **1** and **3** represent the anomeric carbons. The relatively small difference between these two values was expected in view of the axial orientation of the hydroxyl group at C-3″.[20] A 3.8–5.2 ppm change in the anomeric carbon shift is noticed in case of the four other epimeric hexoses. While the quaternary C-3″ signal can be assigned by a SFORD experiment, differentiation of the oxymethine signals C-4″ and C-5″ is made on the basis of the expected identical shift of C-4″ for the two anomers **1** and **3.** The OCH_3″ group of **3** interacts 1,3 diaxially with the axial hydrogen at C-5″, resulting in a shielding of this carbon with respect to C-5″ of **1.** The relatively low-field shift of C-7″ shows the equatorial orientation of this methyl group.[21] Assignment of the remaining carbon signals is straightforward and is based on chemical-shift rules.[22] The isovaleryl side-chain carbon shifts are in agreement with calculated values.[22]

Because of the circumstances mentioned above, an unambiguous analysis of the ^{13}C NMR spectrum of the mixture of β- and α-methyl mycaminosides **5** and **6** could not be achieved. Assignments given in Table I in the 68–71 ppm region for these two compounds are only tentative. The 104.6 and 99.4 ppm shifts represent C-1′ of the β and α anomers, respectively. Among the eight methine signals of the 67–74 ppm region, only two could be attributed with

Table I. ^{13}C Chemical Shifts

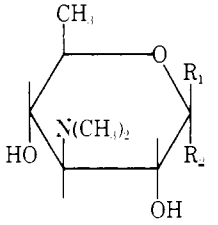
	1	2	3	4	5	6	7	8	9	11	13	14	15	16
C-1	99.4	99.4	98.5	98.6	104.6	99.4	104.0	100.1	101.3	97.5	96.1	102.9	91.0	97.7
C-2	43.3	43.3	40.9	41.5	70.5[a]	70.2[a]	74.7	73.0	82.1	79.7	32.7	31.1	30.2	30.1
C-3	70.9	70.8	69.9	69.8	70.5[a]	67.5	80.2	77.7	79.0	77.7	18.3	18.3	14.4	14.9
C-4	76.7	77.2	76.6	77.0	68.2[a]	68.7[a]	37.4	37.4	72.9	72.1	64.9	65.1	65.6	65.5
C-5	70.3	68.0	65.6	62.9	73.1	69.8[a]	68.0	64.0	70.8	64.3	74.0	73.8	66.9	66.9
C-6	18.1	17.7	18.0	17.5	17.9	17.9	21.0	21.0	17.7	17.4	19.0	18.9	19.0	18.9
C-7	27.3	27.3	25.7	25.9	41.5	41.5					40.7	40.7	40.7	40.7
C-8		172.0		172.7	41.5	41.5					40.7	40.7	40.7	40.7
C-9		43.3		43.4										
C-10		25.6		25.6										
C 11		22.3		22.4										
C-12		22.3		22.4										
C-1 OCH_3	56.4	56.4	55.0	55.0	56.8	54.9	56.9	55.2	56.6	56.1		56.2		54.5
C-2 OCH_3									58.9	57.7				
C-3 OCH_3							56.9	56.9	61.4	61.6				

[a] Assignments may be reversed although those given here are preferred. 5 and 6 were examined as anomeric mixtures. In the text, the prime symbols are applied for convenience to the mycaminose and chalcose, the double prime symbols to the mycarose, and the triple prime symbols to the mycinose and forosamine carbons. The isovaleryl side-chain carbons of 2 and 4 are numbered from C-8 to the end of the chain (C-11 and C-12). The $N(CH_3)_2$ carbons of the mycaminosides 5 and 6 and of the forosamines and forosaminides 13, 14, 15, and 16 are numbered C-7 and C-8.



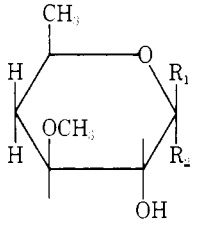
L-mycarose

1, $R_1 = OCH_3$; $R_2 = H$; $R_3 = H$
2, $R_1 = OCH_3$; $R_2 = H$; $R_3 = COCH_2CH(CH_3)_2$
3, $R_1 = H$; $R_2 = OCH_3$; $R_3 = H$
4, $R_1 = H$; $R_2 = OCH_3$; $R_3 = COCH_2CH(CH_3)_2$



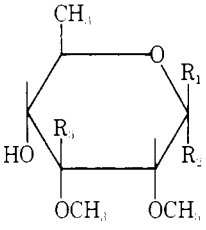
D-mycaminose

5, $R_1 = OCH_3$; $R_2 = H$
6, $R_1 = H$; $R_2 = OCH_3$



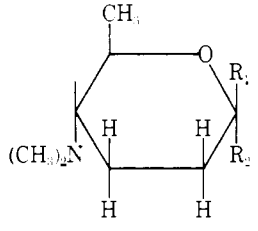
D-chalcose

7, $R_1 = OCH_3$; $R_2 = H$
8, $R_1 = H$; $R_2 = OCH_3$



D-mycinose

9, $R_1 = OCH_3$; $R_2 = H$; $R_3 = H$
10, $R_1 = OCH_3$; $R_2 = H$; $R_3 = D$
11, $R_1 = H$; $R_2 = OCH_3$; $R_3 = H$
12, $R_1 = H$; $R_2 = OCH_3$; $R_3 = D$

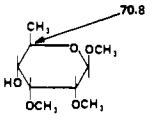


D-forosamine

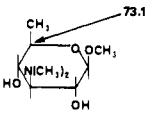
13, $R_1 = OH$; $R_2 = H$
14, $R_1 = OCH_3$; $R_2 = H$
15, $R_1 = H$; $R_2 = OH$
16, $R_1 = H$; $R_2 = OCH_3$

Figure 1.

certainty. The lowest field resonance at 73.1 ppm must be due to C-3′ or C-5′ of the β anomer since the next lowest field signal occurs by 2.6 ppm higher field at 70.5 ppm. While only a small chemical-shift change is noticed for C-2′ and C-4′ between the two compounds, the C-3′ and C-5′ signals are expected to appear a few parts per million higher field in the α with respect to the β anomer. It follows from the same considerations that the highest field resonance at 67.5 ppm must be assigned to C-3′ or C-5′ of the α anomer. An analysis of the ^{13}C NMR spectrum (vide infra) of β-methyl mycinoside **9** permitted a choice between these two possibilities. Since the C-5‴ signal of β-methyl mycinoside **9** could be assigned unambiguously to the 70.8 ppm reso-



9

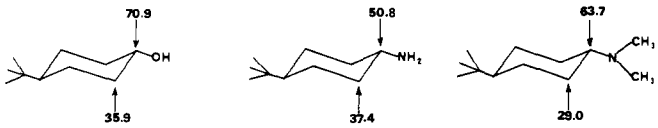


5

nance, the same carbon in β-methyl mycaminoside **5** is expected to appear at lower fields, by a few parts per million, because of the equatorial orientation of its substituent at C-3′. As a consequence, the 73.1 and 67.5 ppm signals were assigned to the C-5′ and C-3′ carbons of the β and α anomers, respectively. These assignments are well supported by an inspection of the ^{13}C NMR spectrum of the various mycaminose containing antibiotics. In Leucomycin-A_3 **17**, for instance, in which mycaminose is present in its β configuration, the 72.9 ppm resonance should be attributed (vide infra) to its aminohexose moiety. The C-4′ signal of mycaminose in the spectrum of this antibiotic appears at 76.0 ppm (vide infra) which would suggest that the 68.2 ppm signal of β-methyl mycaminoside **5** may be due to its C-4′ carbon, in agreement with the strong deshielding influence imposed on the carbon site of attachment of a glycosyl unit in disaccharides.[26] On the other hand, no resonance occurs in the ^{13}C NMR spectrum of **17** between 63.5 and 68.8 ppm, indicating that the 67.5 ppm signal of the anomeric mixture of the mycaminosides **5** and **6** represents a carbon atom of the α anomer. Furthermore chemical shifts due to the hexose moiety of Cirramycin-A_1 **24** (vide infra) (see Table III) support also the given interpretation of the data of the mycaminosides **5** and **6**. While assignments of six signals in the considered region is only tentative, it represents the best fit with chemical-shift rules.[22] Assignment of the higher field methyl signals was straightforward.

It is of interest to note that the C-3′ signal of the mycaminosides appears in the region of the spectrum characteristic of oxymethine carbons. While the result of replacement of an equatorial hydroxyl group by an amino function is an approximately 20-ppm shift of the signal of the substituent carbon toward the higher field, the effect on its immediate

neighbors is small.[23] This effect becomes very different if an *N*-dimethylamino function is the new substituent. A study of 1-substituted 4-*tert*-butylcyclohexane models[24]



shows that this difference is only about 7 ppm for the substituent carbon, while it becomes about 7 ppm toward the higher field for its neighbors.

The 104.0 and 100.1 ppm signals represent the anomeric carbons of β- and α-methyl chalcoside **7** and **8,** respectively. Since a difference in the orientation of the anomeric substituent is expected to cause a greater change of chemical shift for C-3′ and C-5′ than for C-2′, assignment of the oxymethine carbon signals is easy. Because of the expected strong deshielding influence of the methyl of the OCH_3′ group by its β effect on C-3′, the 80.2 and 77.7 ppm shifts are assigned to this carbon of the β and α anomers, respectively. A 1,3-type diaxial interaction between the axial hydrogen at C-5′ and the axial substituent of the anomeric carbon in **8** results in a 4.0 ppm shielding of the C-5′ resonance (64.0 ppm) with respect to the position of this carbon in β-methyl chalcoside **7** (68.0 ppm). The remaining low-field signals at 74.7 and 73.0 ppm were assigned to the C-2′ carbons on the basis of the large intensity difference that they exhibited. This is in agreement with the known proportion of the constituents in the anomeric mixture. A lower field signal for C-2′ in the case of the β anomer is reasonable in view of the greater β effect of an equatorial substituent with respect to an axial one.

Besides the low-field anomeric carbon signals, the four oxymethine resonances of each of the methyl mycinosides **9** and **11** were assigned as follows. While the lowest field ones at 82.1, 79.7, 79.0, and 77.7 ppm must be due to C-2‴ and C-3‴ because of the known deshielding influence (vide supra) of the methyls attached to the oxygen atoms, the 72.9 and 72.1 ppm resonances were assigned to C-4‴ of the samples separately examined. C-4‴ in the two compounds is not expected to exhibit a significant chemical-shift difference, while C-5‴ of α-methyl mycinoside **11** should be strongly shielded with respect to the β anomer **9** because of a 1,3-diaxial interaction in which C-5‴ is involved in case of **11.** As a consequence, the 70.8 and 64.3 ppm resonances must represent the C-5‴ carbon of the β and α anomers **9** and **11,** respectively. Since the differentiation of the C-2‴ and C-3‴ signals could not be done on the basis of these spectra, the corresponding deuterio derivatives at C-3‴, **10** and **12,** were prepared.[17a] The ^{13}C NMR spectra of **10** and **12** were identical with those of **9** and **11** except that the 79.0 and 77.7 ppm signals were missing. Thus a complete assignment of the methyl mycinosides **9** and **11** was achieved.

A precise analysis of the spectrum of the fifth antibiotic sugar component forosamines **13** and **15** and their derivatives **14** and **16** was easy. Differentiation between the C-4‴ and C-5‴ resonances of these compounds was done on the basis of the expected little variation of the signal due to the former carbon. Of the two methylene resonances due to C-2‴ and C-3‴, the higher field one was assigned to C-3 as a consequence of the γ effect of both methyl carbons attached to the nitrogen atom. This assignment was well supported by the upfield shift of the C-3‴ signal in the α as compared with the β anomers. All the other assignments of these compounds were straightforward and do not require special comments.

Spectral Analysis of the Antibiotics. From the point of view of the spectral analysis, the 16-membered macrolide antibiotics that were studied (Figure 3) can be divided into two classes. Table II contains the chemical-shift assignments for a number of structurally related compounds, all containing the mycarosyl-mycaminose disaccharide moiety. For the sake of clarity in the presentation of the results, chemical-shift data along with the specific assignments for Tylosin **23,** Cirramycin-A_1 **24,** and for the two neutral substances Chalcomycin **25** and Neutramycin **26** were indicated separately in Table III. The spectral analysis of the antibiotics was greatly aided by the spectra of their sugar components described in the preceding paragraphs (Table I).

The low-field part of the spectra of the compounds presented in Table II contains the resonances due to the lactone carbonyl at C-1, the keto carbonyl at C-9, the unsaturated carbons from C-10 to C-13, the aldehyde carbonyl at C-18, and that due to the acetate or propionate carbonyl at C-20. The lowest field signal among them represents the aldehyde carbonyl at 201.2 and 202.7 ppm in Leucomycin-A_3 **17** and A_5 **18,** respectively. In Magnamycin-A **21,** C-9 and C-18 are overlapping signals while, in Magnamycin-B **22,** these resonances are resolved but are still too close to each other for an unambiguous assignment to be made. Two further resonances appear at higher field and close to one another. The one at 170.0 ± 0.2 ppm in those compounds having an acetate group at C-3 was assigned to the lactone carbonyl at C-1 and the other (170.8 and 173.8 ppm in **17** and **20,** respectively) to the acetate or propionate carbonyl at C-20.

It is interesting to note that the chemical shift of the lactone carbonyl of Leucomycin-A_5 **18,** Tylosin **23,** and Cirramycin-A_1 **24** (see Table III) is significantly deshielded (about 4 ppm) with respect to the resonance position of C-1 in **17, 20, 21,** and **22.** In the case of the former compounds, the hydroxyl group at C-3 is not esterified, and the carbonyl deshielding is interpreted as the consequence of an intramolecular hydrogen bonding as depicted in Figure 2. Similar conclusions were reached on the basis of ir studies about the previously reported conformational analysis of Leucomycins.[8b]

The four ethylenic carbons of the Leucomycins **17** and **18** and Spiramycin III **20** resonate between 126 and 136 ppm. Specific hydrogen decoupling experiments help to assign C-10 and C-11, while C-12 and C-13 cannot be differentiated.[25] In the case of the Magnamycins **21** and **22,** C-11 must be assigned to the lowest and C-10 to the highest field signal as a consequence of the known effects resulting from double-bond conjugation with a carbonyl group.[22]

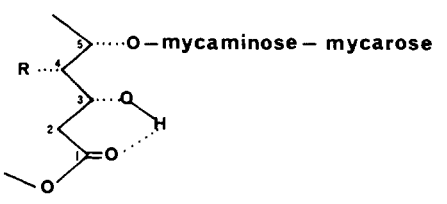
The region of the spectra around 100 ppm, characteristic of the anomeric carbon resonances of the sugar components, was easy to analyze in view of the previously described ^{13}C NMR study of the hexose models. The 103.5 ± 0.5 ppm signal was assigned to C-1′ of the mycaminose moiety, indicating the β configuration of this sugar in all the antibiotics, in agreement with previous studies[3] and with the C-1′ shift at 104.6 ppm of β-methyl mycaminoside **5** (Table I). The 96.7 ± 0.3 ppm resonance was characteristic of C-1″ of the mycarose part of all the substances investigated. Thus the 100.3 ppm signal of Spiramycin III **20** had to be assigned to the anomeric carbon of its forosamine moiety.

Unambiguous chemical-shift assignments between 57 and 87 ppm presented challenging problems in view of the high number of resonances that this region of the spectra revealed. In the ^{13}C NMR spectrum of Leucomycin-A_3 **17,** for instance, the 68–78 ppm region contained ten resolved carbon signals. Most resonances were assigned by comparison with the spectra of the model hexoses (Table I). An excellent agreement was observed between the chemical shifts

Table II. ^{13}C Chemical Shifts

	17	18	19	20	21	22
C-1	169.9	173.5[b]	170.0[b]	169.9	170.0	169.9
C-2	37.0	38.0	36.9	37.2	37.2	36.7
C-3	71.6	71.7	68.9	71.7	71.2	71.3
C-4	77.5	79.2	80.8[c]	77.8	77.2	77.7
C-5	84.9	85.2	80.0[c]	84.5	84.4	84.5
C-6	28.8[b]	30.7	38.5[d]	28.9[b]	30.4[b]	30.9[b]
C-7	30.4[b]	30.7	31.7	30.2[b]	31.0[b]	32.1[b]
C-8	33.5	34.1	36.9[d]	31.9	39.0	40.6
C-9	73.1	73.0	73.5	79.7	199.3	201.9[c]
C-10	127.6	130.2	128.7	126.7	124.3	121.8
C-11	135.7	134.1	133.7	135.4	146.0	143.6
C-12	132.6[c]	132.5[c]	131.7[e]	132.3[c]	59.0[c]	132.0
C-13	132.1[c]	131.5[c]	131.3[e]	131.9[c]	57.8[c]	139.8
C-14	40.9[d]	41.9[d]	41.0	40.6[d]	42.9[d]	42.9[d]
C-15	68.8[e]	68.3[e]	68.9	68.9[e]	68.5[e]	68.2[e]
C-16	20.3	20.1	20.3	20.3	20.5	20.4
C-17	42.4[d]	43.1[d]	35.4[d]	42.5[d]	44.1[d]	44.0[d]
C-18	201.2	202.7	97.2	201.3	199.3	200.0[c]
C-19	14.7	15.0	15.1	15.4	17.1	17.3
C-20	170.8		169.5[b]	170.8	168.9	168.9
C-21	21.3		21.0	27.7	20.8	21.0
C-21[a]				9.0		
C-22	62.4	61.7	60.9	62.4	62.1	62.1
C-1′	103.7	104.0		104.0	103.1	103.3
C-2′	69.0[e]	68.8[e]		69.2[e]	68.5[e]	68.5[e]
C-3′	69.0[e]	69.1[e]		69.2[e]	69.1[e]	68.8[e]
C-4′	76.0	76.0		74.9	76.0	75.8
C-5′	72.9	73.0		73.0	72.7	72.7
C-6′	18.8[f]	18.8[f]		19.0[f]	18.7[f]	18.7[f]
C-7′	41.9	41.9		42.0	41.7	41.7
C-8′	41.9	41.9		42.0	41.7	41.7
C-1″	97.0	97.0		96.4	96.8	96.7
C-2″	41.9	41.9		41.0	41.7	41.7
C-3″	69.3	69.3		69.5	69.1	69.1
C-4″	77.1	77.2		76.4	76.8	76.8
C-5″	63.5	63.5		66.1	63.4	63.3
C-6″	17.8[f]	17.8[f]		18.3[f]	17.7[f]	17.7[f]
C-7″	25.5	25.3		25.4	25.4	25.4
C-8″	172.9	173.9[b]			172.1	172.1
C-9″	43.3	36.2			43.2	43.1
C-10″	25.5	18.5			25.4	25.4
C-11″	22.4	13.6			22.3	22.3
C-12″	22.4				22.3	22.3
C-1‴				100.3		
C-2‴				31.2		
C-3‴				18.3		
C-4‴				64.9		
C-5‴				73.7		
C-6‴				18.7[f]		
C-7‴				41.0		
C-8‴				41.0		

[a-f] Assignments within any vertical column may be reversed. In view of the small quantities available, SFORD experiments were not run on 19, 21, and 22. The prime symbols are applied for convenience to the mycaminose, the double prime symbols to the mycarose, and the triple prime symbols to the forosamine carbons.



18 R = OCH_3

23 R = CH_3

24 R = CH_3

Figure 2.

of the hexose models and those of the sugar moieties of the antibiotics. In the case of Spiramycin III **20**, where the configuration of the linkage of the forosamine moiety was not yet known, both β- and α-methyl forosaminides **14** and **16** were considered as model compounds.

In addition to the signals of β-methyl mycaminoside **5** and α-methyl 4-*O*-isovaleryl mycaroside **4** which could be assigned by such straightforward comparisons, the spectrum of Leucomycin-A_3 **17** exhibited seven resonances in this region. The C-4′ signals of the antibiotics would be expected to be deshielded relative to the model β-methyl mycaminoside **5** because of the linkage at this position of the sugars in the disaccharide unit.[26] The influence of such a linkage on the chemical shift of the neighboring carbons, in

Table III. ^{13}C Chemical Shifts

	23	24		25		26
C-1	173.9	173.5		164.7		165.3
C-2	39.4	39.7		120.3		120.9
C-3	71.7	71.1		151.0		151.0
C-4	45.1	45.2		41.5		40.7
C-5	81.6	81.3		87.5		82.0
C-6	32.3[a]	31.8		36.7		30.9
C-7	32.9[a]	31.8		33.9		26.5[a]
C-8	40.3	37.8		78.3		78.4
C-9	202.8[b]	200.2		199.4		200.4
C-10	118.8	122.9		124.5		124.6
C-11	148.0	151.0		145.8		146.5
C-12	134.9	59.7		59.4[a]		59.1
C-13	142.2	67.1		58.8[a]		59.1
C-14	44.7	40.9		49.4		49.5
C-15	75.3	77.1		68.5[b]		68.9
C-16	25.5	24.8		18.3[c]		18.1
C-17	43.9	43.9		18.5[c]		
C-18	203.0[b]	202.5				
C-19	17.4	17.5		27.7		26.0[a]
C-22	9.6[c]	9.3[a]		19.1[c]		18.1
C-23	13.0	15.1[b]				
C-24	68.2	14.5[b]		66.7		67.1
C-25	9.0[c]	9.0[a]				
C-1′	103.9	104.2		102.9		103.9
C-2′	69.5	70.3		74.8		74.9
C-3′	69.5	71.1		80.3		80.3
C-4′	75.3	68.0		36.7		36.9
C-5′	73.2	73.5		67.5[b]		68.1
C-6′	19.0[d]	17.8		20.8		20.9
C-7′	42.0	41.7	C-3′ OCH_3	56.8	C-3′ OCH_3	56.8
C-8′	42.0	41.7				
C-1″	96.6					
C-2″	41.1					
C-3″	69.0					
C-4″	76.5					
C-5″	66.1					
C-6″	18.3[d]					
C-7″	25.5					
C-1‴	101.1			100.5		100.9
C-2‴	82.0			81.6		81.5
C-3‴	79.9			79.4		79.7
C-4‴	72.9			72.5		72.8
C-5‴	70.6			70.4		70.7
C-6‴	17.8[d]			17.7		18.1
C-2‴ OCH_3	59.6			58.5		59.1
C-3‴ OCH_3	61.7			61.4		61.7

[a-d] Assignments within any vertical column may be reversed. The prime symbols are applied for convenience to the mycaminose (for 23 and 24) and chalcose (for 25 and 26) carbons. The double prime and triple prime symbols are applied for the mycarose and mycinose carbons, respectively.

this case on C-3′ and C-5′, would be expected from previous studies to be small.[26] Thus these seven signals from low field to high field at 84.9, 77.5, 76.0, 73.1, 71.6, 68.8, and 62.4 ppm had to be assigned to the C-3, C-4, C-5, C-9, C-15, C-22, and C-4′ carbon atoms in an order which was to be decided. Except for Leuconolide-A_3 5,18-hemiacetal **19**,[27] which may have a markedly different conformation compared with the other substances of Table II, a 84.8 ± 0.4 ppm signal was observed in the spectra of the Leucomycins **17** and **18**, Spiramycin III **20**, and the Magnamycins **21** and **22**. Because of the strong deshielding β effect of C-1′ and that of C-22 on the chemical shifts of C-5 and C-4, respectively, the 84.9 ppm resonance of Leucomycin-A_3 **17** had to be assigned to either of these carbons. On the other hand, a 77.5 ± 0.3 ppm signal was present in the ^{13}C NMR spectra of all these antibiotics except Leucomycin-A_5 **18** in which this resonance appeared at 79.2 ppm. This small deshielding effect associated with the absence of an acetyl group in Leucomycin-A_5 **18** was interpreted as characteristic of the C-4 carbon. As a result, the 84.9 ppm signal was assigned to C-5, and the remaining lowfield resonance at 76.0 ppm was attributed to the C-4′ carbon of Leucomycin-A_3 **17**. This interpretation of the results was in good agreement with the ^{13}C NMR spectra of Tylosin **23** and Cirramycin-A_1 **24** (vide infra). While the 77.5 ± 0.3 ppm resonance was absent in the spectra of these two compounds (Table III), C-4′ in Tylosin **23** appeared at 75.3 ppm and in Cirramycin-A_1 **24**, in the absence of the mycarose moiety, at 68.0 ppm in accord with the observed spectrum of β-methyl mycaminoside **5**.

The 73.1 ppm resonance of Leucomycin-A_3 **17** was assigned to C-9 since this carbon had the same chemical shift in Leucomycin-A_5 while, in Spiramycin III **20**, it was deshielded by 6.6 ppm as a consequence of the attachment at this site of the forosamine moiety. In the Magnamycins **21** and **22**, this resonance was replaced by the low-field carbonyl signal. The C-22 carbon was attributed to the highest field resonance in this region, at 62.4 ppm in the spectrum of Leucomycin-A_3 **17**, and was confirmed by a SFORD experiment. A choice between the remaining two signals rep-

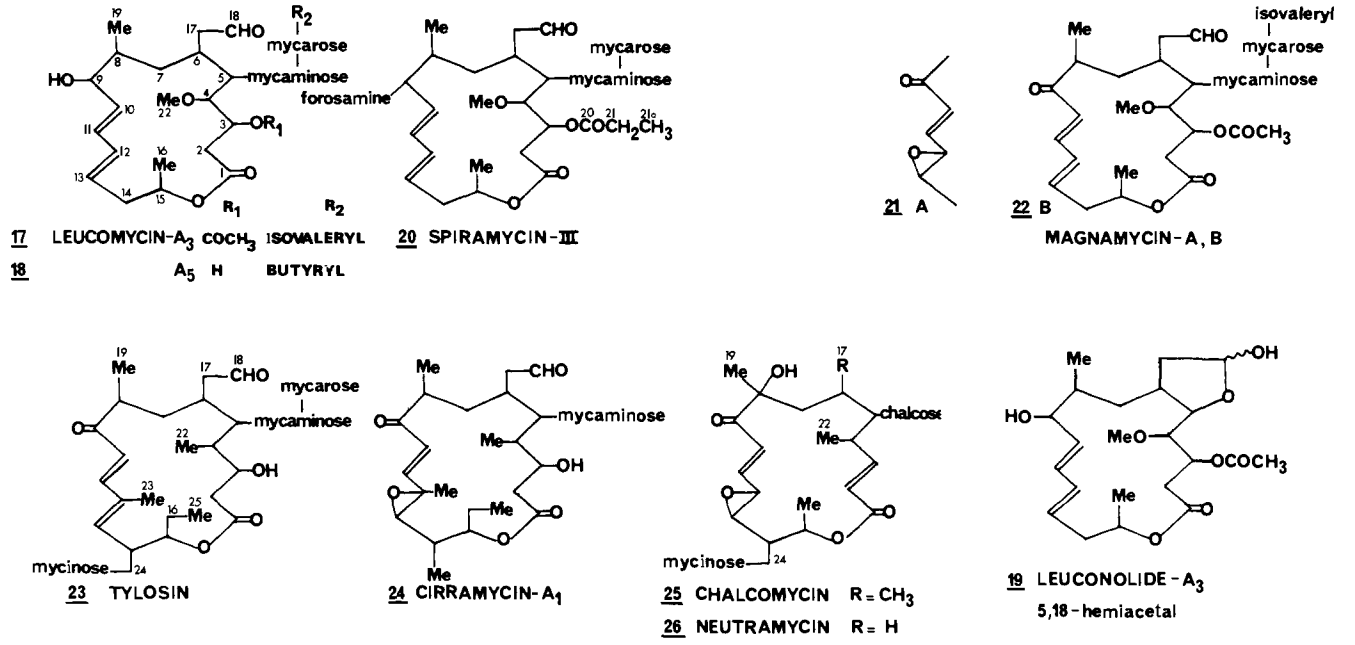


Figure 3.

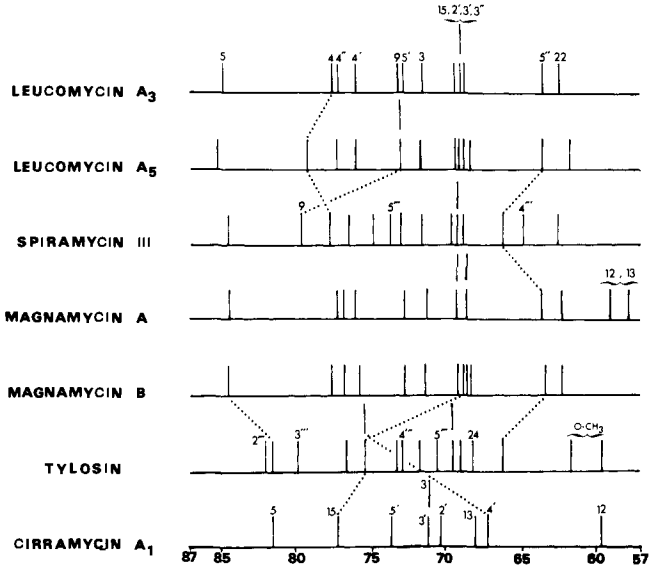


Figure 4. ^{13}C chemical shift correlation diagram.

resenting C-3 and C-15 was made again by comparison with the spectra of Tylosin **23** and Cirramycin-A_1 **24.** These two carbon signals appeared for all the compounds of Table II, even in the spectrum of Leucomycin-A_5, at 71.5 ± 0.3 and 68.4 ± 0.4 ppm. Thus no acetylation effect on C-3 could be detected. The 71.6 ppm signal of Leucomycin-A_3 was assigned to C-3 and the 68.8 ppm resonance to C-15 on the basis of the presence and absence respectively of such signals in the spectra of Tylosin **23** and Cirramycin-A_1 **24.** The higher field epoxy carbon resonances of Magnamycin-A **21** at 59.0 and 57.8 ppm, due to its C-12 and C-13, could not be differentiated. The ^{13}C NMR spectrum of Leuconolide-A_3 5,18-hemiacetal **19,** isolated from Leucomycin-A_3 by a published procedure,[23] was also recorded. Chemical-shift assignments for this compound were based on the spectra of the Leucomycins **17** and **18** and will not be discussed. A correlation diagram of ^{13}C shifts in the 57–87 ppm region for some of the antibiotics studied is shown in Figure 4, and Figure 5 indicates the ^{13}C NMR spectrum of Spiramycin III **20,** a representative member of the macrolides considered.

The 44–57 ppm region of the various spectra did not contain any resonance, whereas the 39–44 ppm section exhibited five to seven signals, depending on the structure of the antibiotic. Some of these resonances were due to the hexose carbons and could be recognized readily by comparison with the chemical shifts of the appropriate models in Table I. Thus the C-7′, C-8′, and C-2″ carbons of **17, 18, 20, 21,** and **22,** the C-9″ carbon of **17, 21,** and **22,** and the C-7‴ and C-8‴ carbons of **20** appeared in the considered region. Besides these easily assignable four signals, Leucomycin-A_3 **17** showed two additional resonances in the 39–44 ppm area of its spectrum. Although their differentiation was not unequivocal, these two resonances at 42.4 and 40.9 ppm were assigned to C-17 and C-14. This interpretation of the results is based on the SFORD spectrum of **17,** on the alkyl carbon shieldings of some linear aldehydes[28] as well as on the following considerations. The 28–37 ppm region of the Leucomycin-A_3 **17** spectrum exhibited four signals. While three of them showed only a little variation (1–2 ppm) in the spectra of **17, 18, 20, 21,** and **22,** the 33.5 ppm signal of **17** was strongly deshielded in the Magnamycins **21** and **22** as a consequence of the influence of the neighboring keto group at C-9. Thus the 33.5 ppm signal of Leucomycin-A_3 **17,** showing a doublet in its SFORD spectrum, was assigned to C-8. Among the other three signals of the 28–37 ppm section (C-7, C-6, and C-2), C-2 would be expected to resonate at lower field than both C-6 and C-7. This hypothesis was based on the nature of the neighboring sites of C-2 which are the lactone carbonyl and the acetoxy group at C-3. Inspection of the spectra of **17** and **18** indicated that two signals of the considered spectral region were slightly deshielded as a result of the loss of the acetate group at C-3. Thus the shift contrast between **17** and **18** could not unequivocally confirm the 37.0 ppm signal assignment for C-2 in the spectrum of **17.** However, the SFORD spectrum indicated clearly that the 37.0 ppm signal of **17** was a methylene type carbon and, as a consequence, it could represent C-2 or C-7. Its final assignment to C-2 was based on the shift contrast between the ^{13}C NMR spectrum of Chalcomycin **25** and Neutramycin **26.** In the spectrum of Chalcomy-

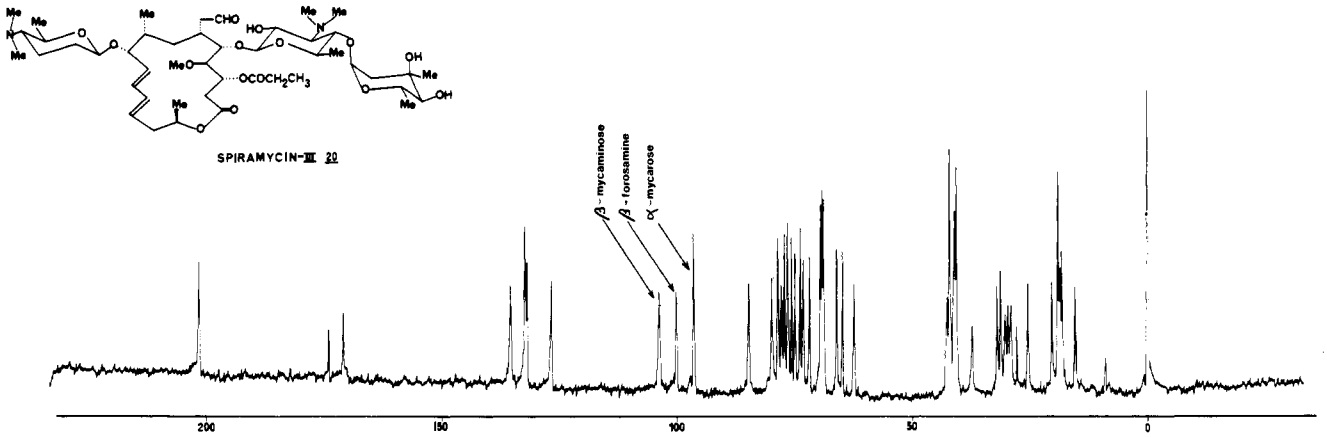


Figure 5. ^{13}C spectrum and structure of Spiramycin III.

cin **25**, the C-7 signal (33.9 ppm) could be assigned unambiguously by comparison with Neutramycin **26** (C-7; 26.5 ppm) because of the absence of the C-17 carbon in the latter. Since in the light of chemical-shift rules,[22] it is reasonable to suppose that C-7 of **17** should appear high field with respect to C-7 of **26**, the 30.4 or 28.8 ppm resonance of Leucomycin-A_3 **17** was assigned to C-7. If the 30.4 ppm signal represents C-7, the remaining 28.8 ppm resonance corresponds to C-6 whose multiplicity in the SFORD spectrum, though not absolutely unequivocally, indicates a doublet.[30]

The question arose whether C-17 and C-14 were not mistakenly assigned to the signals of the 39–44 ppm region, and calculated values in terms of the Grant rules[29] for C-3 substituted propanal derivatives[28] indicated that C-17 should have a chemical shift over 40 ppm. As far as the C-14 signal is concerned, its resonance position over 40 ppm in **17** will be justified by an inspection of the spectra of the compounds presented in Table III.

An additional signal at 36.2 ppm appeared in the considered section of the spectrum of Leucomycin-A_5 **18.** Since in this compound a butyryl side chain is attached to its mycarose moiety, the 36.2 ppm resonance was assigned to C-9″ of **18** in agreement with the 35.8 ppm signal of C-2 of methyl butyrate[22] **27.** The two higher field signals of **18** at 18.5 and 13.6 ppm could be explained in the same way (Figure 6).

The interpretation of the remaining high-field part (0–27 ppm) of the ^{13}C NMR spectrum of Leucomycin-A_3 **17** was rather straightforward. Six of the nine signals of this area of the spectrum were representative of the sugar moieties (see Tables I and II). The 21.3 ppm resonance was assigned to C-21 since this signal was absent in the spectrum of Leucomycin-A_5 **18** and replaced by a 27.7 ppm signal in the spectrum of Spiramycin III as a consequence of an ethyl ester at C-3 in **20.** The 14.7 ppm signal was assigned to C-19 as a result of the shift contrast between the spectra of the compounds of Table II. In the spectra of the Magnamycins **21** and **22,** the C-19 resonance was deshielded by the effect of the replacement of a hydroxyl group by a keto function in γ position to it. The remaining resonance at 20.3 ppm was attributed to the last methyl carbon (C-16) which was not yet assigned and which showed only a little variation throughout the whole series.

The spectral analysis of the compounds shown in Table III requires a few additional comments, although a discussion in connection with some of the carbon assignments was already presented above. With respect to Tylosin **23,** the C-5 carbon of Chalcomycin **25** was deshielded by 5.9 ppm in conformity with the loss of two γ effects, although the homoallylic endocyclic influence[31] of the Δ^2 double bond

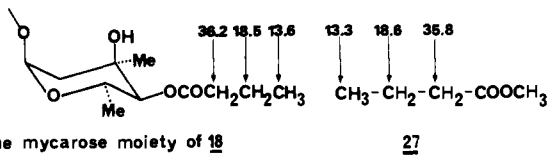


Figure 6.

could not be evaluated. The 75.3 ppm signal in the spectrum of Tylosin **23** was assigned to C-15 since due to two additional deshielding effects this carbon was expected to appear at low field as compared with the spectrum of Leucomycin-A_3 **17** (C-15; 68.8 ppm). It is interesting that the easily assigned C-15 carbon in Chalcomycin **25** has approximately the same shift as in Magnamycin-A in spite of the trisubstituted C-14 carbon in **25.** This fact may reflect significant conformational changes for the respective macrocyclic portions of **21** and **25.**

The shift differences for C-5, C-6, and C-7 between Chalcomycin **25** and Neutramycin **26** allowed a precise evaluation of α and β effects because of methyl substitution on the 16-membered rings. While the α effect is +5.8 ppm, the two β effects are +5.5 and +7.4 ppm.

In the spectrum of Tylosin **23,** two methine resonances can be noticed at 45.1 and 44.7 ppm. These signals were assigned to C-4 and C-14, respectively. This interpretation of the data is well supported by the spectrum of Cirramycin-A_1 **24** in which the corresponding carbons resonate at 45.2 and 40.9 ppm. As expected, C-14 of **24** is shielded with respect to C-14 of **23.** Considering the structures of **17** and **24** and the various α, β and γ effects influencing the chemical shift of their C-14 signal, full justification can be found for the previously assigned C-14 resonance of **17** over 40 ppm. Known models[22] suggested that the 25.5 ppm signal in the spectrum of **23** should be assigned to the highest field methylene (C-16), while the methyl carbons C-22 and C-25 could not be differentiated. The same negative conclusion was reached in connection with C-23 and C-24 of Cirramycin-A_1 **24.**

Structural and Conformational Implications of the Signal Assignments

The Structure of the Spiramycins. In spite of a series of reports on the structure of the Spiramycins,[8a-c] the anomeric configuration linking the forosamine portion to the aglycone was not known. Evidence is presented in this study for the β configuration of this center, and Figure 5 shows the complete structure and the ^{13}C NMR spectrum of Spiramycin III **20.**

On the basis of Tables I and II, chemical-shift assign-

Table IV. ^{13}C Chemical Shifts

	Forosamine moiety of Spiramycin III 20	β-Methyl forosaminide 14	α-Methyl forosaminide 16
C-1‴	100.3	102.9	97.7
C-2‴	31.2	31.1	30.1
C-3‴	18.3	18.3	14.9
C-4‴	64.9	65.1	65.5
C-5‴	73.7	73.8	66.9
C-6‴[a]	18.7	18.9	18.9
C-7‴	41.0	40.7	40.7
C-8‴	41.0	40.7	40.7
	OCH_3‴	56.2 OCH_3‴	54.5

[a] This carbon may be assigned to either of the signals 18.3, 18.7, and 19.0 ppm in the spectrum of 20 (see Table I).

Table V. ^{13}C Chemical Shifts and $J_{^{13}C-^1H}$ Coupling Constants (±2 Hz) for Some Anomeric Carbons

Spiramycin III 20	
C-1′ (β-mycaminose)	104.0 ppm; 158.4 Hz
C-1″ (α-mycarose)	96.4 ppm; 167.5 Hz
C-1‴ (β-forosamine)	100.3 ppm; 158.4 Hz
β-Methyl mycaroside 1	99.4 ppm; 161.9 Hz
α-Methyl mycaroside 3	98.5 ppm; 168.1 Hz
β-Forosamine 13	96.1 ppm; 157.7 Hz
α-Forosamine 15	91.0 ppm; 165.1 Hz

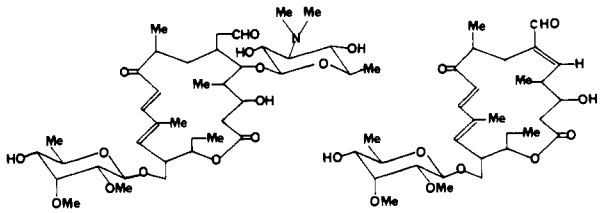
ments for the forosamine moiety of Spiramycin III **20**, β- and α-methyl forosaminide **14** and **16**, respectively are shown in Table IV.

Inspection of the data presented in Table IV shows clearly the β configuration of the anomeric center of the forosamine portion of Spiramycin III **20**. The C-1‴ shift of **20** at 100.3 ppm is a little shielded with respect to that of **14** as a consequence of the γ effects on C-1‴ of the C-8 and C-10 carbons of the lactone ring. On the other hand, both the structurally diagnostic signals C-3‴ and C-5‴ of **20** are identical with the shifts of the corresponding carbons of **14**. In the case of α-methyl forosaminide **16**, these resonances are strongly shielded as a result of 1,3-diaxial interactions of the axial hydrogens at C-3‴ and C-5‴ with the axially oriented anomeric substituent.

Our conclusion about the β configuration of the forosamine moiety in the Spiramycins was corroborated by another approach to the problem. Recent reports in carbohydrate chemistry have indicated a clear dependence of the anomeric coupling constant $J_{^{13}C-^1H}$ on the orientation of the substituent of C-1. Perlin and Casu[32] and later Bock, Lundt, and Pedersen[33] found that hexoses in which H_1 is axially oriented have a $J_{^{13}C-^1H}$ value of 158–162 Hz, whereas the hexoses with an equatorial H_1 have higher J values (169–171 Hz). By the use of gated decoupling,[34] we have recorded the ^{13}C NMR spectrum of Spiramycin III **20** and measured the coupling constants for the anomeric carbons of each of its hexose components.

Similar experiments were carried out on the model compounds **1, 3, 13,** and **15,** and the results are presented in Table V. Since it is known that the mycaminose and mycarose moieties of the Spiramycins have β and α configurations, respectively,[8a-c] the anomeric coupling constant of the forosamine part of **20** is in agreement with conclusions reached on the basis of chemical-shift data about the orientation of its substituent at C-1‴.

The Structure of Tylosin 23. The structure of Tylosin[7] **23** has been elucidated previously almost completely. The remaining problem was whether the disaccharide portion was attached to the oxygen atom at C-3 or at C-5. Morin et al.[7] advanced the hypothesis of a C-5 linkage on the basis of the structure of a by-product—mycinosyl anhydronortylonolide (MAT) **28**—resulting from the oxidation of Desmycosin **29**



29 **28**

which was obtained by mild acid hydrolysis of Tylosin **23.** Location of the disaccharide moiety was suggested to be at C-5 in order to explain the genesis of the additional Δ^5 double bond in MAT **28.**

The macrolide structure of Cirramycin-A_1 **24** has been proposed by Tsukiura et al.,[15] and the position of the attachment of its lactone moiety to mycaminose at C-5 was elucidated by Suzuki,[19] by spin-decoupling experiments on the related B-58941 antibiotic.

This paper presents evidence that the mycarosyl mycaminose portion of Tylosin **23** is also attached to its C-5 oxygen atom. Inspection of chemical-shift data shown in Table III indicates practically identical resonance positions for all carbons from C-1 to C-6 for Tylosin **23** and Cirramycin-A_1 **24.** In the light of chemical-shift rules,[22] this fact cannot be reconciled with a C-5 sugar attachment in **24** and a C-3 attachment for Tylosin **23.**

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Alkyl Metal Asymmetric Reduction. VII. Temperature Dependence of the Stereoselectivity of Alkyl Phenyl Ketone Reductions by Chiral Organoaluminum Compounds

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Abstract: The reduction of a series of alkyl phenyl ketones by optically active tris(2-methylbutyl)aluminum and its diethyl etherate has been investigated at temperatures ranging from 0 to −60°. The stereoselectivity of the process has been interpreted on the basis of enthalpic and entropic contributions. The suggestions previously proposed for the stereochemistry of this reaction are consistent with the results obtained. The stereochemical course of the reductions by the organoaluminum diethyl etherate confirms that the ligand to the aluminum atom partakes in the transition state of the reaction and controls the extent of stereoselectivity.

Reactions involving hydrogen transfer from chiral organometallic compounds to prochiral functional substrates have been widely investigated in recent years.[1] Stereochemical models which are commonly devised to rationalize such asymmetric reductions do not always imply an accurate knowledge of the mechanistic pathways of the reactions when the reacting species, for example Grignard reagents,[2] have a complex nature.

It is indeed to be considered that schemes of asymmetric induction based on steric requirements are usually oversimplified and that polar[3,4] and solvation effects[4] can assume a remarkable importance.

From this point of view, the use of organoaluminum compounds as chiral reducing agents has appeared suitable for investigating this kind of asymmetric reductions[4] taking into account that such compounds have a defined structure,[5] can be employed both in hydrocarbon and ethereal solvents, and reduce carbonyl substrates through well-established mechanisms.[6] In this connection, to check the picture previously proposed for the stereochemistry of reduction of alkyl phenyl ketones by optically active β-branched organoaluminum compounds,[4a] we have studied the effect of temperature on the stereoselectivity of reduction of alkyl phenyl ketones both by (+)-tris[(*S*)-2-methylbutyl]aluminum[7] (Al2MB) and (+)-tris[(*S*)-2-methylbutyl]aluminum diethyl etherate[8] (Al2MB·OEt_2).

Experimental Section

General. (+)-Tris[(*S*)-2-methylbutyl]aluminum and (+)-tris[(*S*)-2-methylbutyl]aluminum diethyl etherate, prepared as previously reported,[10,3] were carefully redistilled under nitrogen and stored in sealed glass vials.

The ketones employed were obtained from the purification of commercial products; *tert*-butyl phenyl ketone was prepared according to the procedure already mentioned.[4a] Reductions were carried out at 0°, using ice–water baths, and in baths regulated at −30 and −60° (±3°). A Schmidt–Haensch polarimeter was used for all the determinations of the optical rotations. GLC analyses were performed on a C. Erba Fractovap Model GT instrument with flame ionization detectors, using 200 × 0.30 cm columns packed with 10% BDS on 60–80 mesh Chromosorb W at 150°.

Reactions. Reactions were carried out in a flame-dried, two-neck 100-ml flask, fitted with a reflux condenser, a dropping fun-