STUDIES ON THE IONOPHOROUS ANTIBIOTICS. IX[1] THE STRUCTURE OF 4-METHYLSALINOMYCIN (NARASIN)

Sir:

By comparison of the mass spectra of salinomycin and narasin, OCCOLOWITZ *et al.*[2] have recently determined the structure of narasin as 4-methylsalinomycin. We have also arrived at the same conclusion based on the mass spectral analysis of salinomycin[3] and 4-methylsalinomycin.

Mass spectral analysis, however, only established that the substitution by a methyl group on salinomycin to give narasin takes place at C-4 and as in most cases, no experimental evidence with regards to the stereochemical disposition of the new methyl group could be obtained.

We wish to report unequivocal evidence obtained by ^{13}C-nmr spectroscopy that narasin is 4-methylsalinomycin and that the newly introduced methyl group is equatorially oriented (Fig. 1).

In accord with the mass spectral analysis, the ^{13}C-nmr spectrum* of 4-methylsalinomycin (**1**)**, $C_{43}H_{72}O_{11}$, m.p. 103~105°C, M^+ (*m/e*) 764, revealed that a methylene (δ_c 20.1 ppm) in salinomycin (**II**), $C_{42}H_{70}O_{11}$, has been replaced by a methyl (18.9 ppm) and a methine (28.9 ppm) in **I** as shown in Fig. 2. Therefore, the methyl substitution should be at C-4 or C-5.

Although detailed assignment of the ^{13}C-nmr spectrum of salinomycin will be given elsewhere, brief comment is necessary on the partial assignment of **II** which is relevant to this structural work. In the ^{13}C-nmr spectrum of **II** labeled with 1,2-^{13}C propionate were observed two pairs of an AB-type ^{13}C-^{13}C coupling between a methylene and a methine [26.4(CH_2) and 28.0(CH), J_{C-C}= 33 Hz, and 38.6(CH_2) and 40.7(CH), J_{C-C}= 33 Hz]. In view of the chemical shift of the carbons under consideration, these carbon sequences are found only at (C-5)–(C-6) and (C-15)–(C-16). Other possibilities, *i.e.* (C-2)–(C-41), (C-12)–(C-36) and (C-14)–(C-15) are excluded, since C-2 and C-12 have been easily assigned to

Fig. 1. Structure of 4-methylsalinomycin (**I**, R=CH_3) and salinomycin (**II**, R=H)

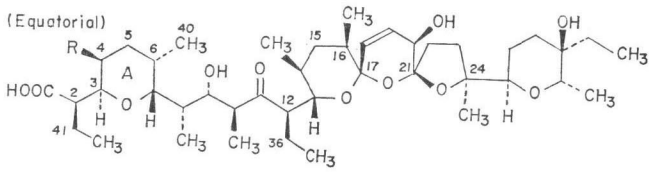
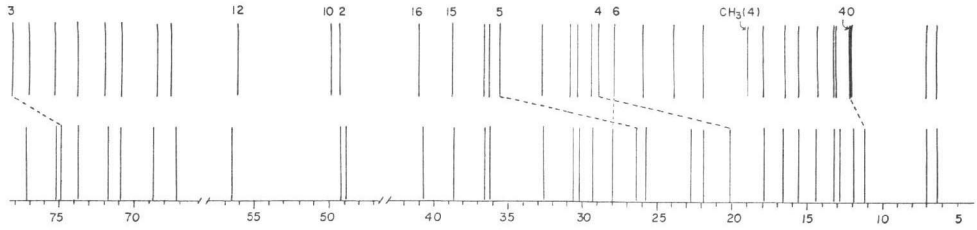


Fig. 2. ^{13}C-Nmr spectra of 4-methylsalinomycin (**I**, upper) and salinomycin (**II**, lower).
25.05 MHz, in $CDCl_3$.
Signals not shown in the figure are as follows:
4-Methylsalinomycin; 88.4 (C-24), 99.5 (C-17), 106.4 (C-21), 121.9 (C-18), 131.9 (C-19), 178.2 (C-1) and 216.4 (C-11).
Salinomycin; 88.5 (C-24), 99.2 (C-17), 106.4 (C-21), 121.6 (C-18), 132.4 (C-19), 177.2 (C-1) and 214.5 (C-11).



* ^{13}C-nmr spectra of **I** and **II** were determined with their free acids. Chemical shifts are expressed in ppm from internal TMS.

** 4-Methylsalinomycin was isolated from *Streptomyces* sp. independently by Kaken Chemical Co. and as C-7819B by Takeda Chemical Industries. The identity of narasin, 4-methylsalinomycin and C-7819B was confirmed by direct comparison of the compounds.

48.9 and 56.5 ppm respectively, based on their chemical shift trends. The experiment using 1,2-^{13}C acetate proved that C-17 and C-18 are derived from the same acetic acid molecule. Therefore, it seems biosynthetically most reasonable to assume that C-15 is coupled to C-16 rather than to C-14 in **II** labeled with 1,2-^{13}C propionate. This assumption, however, is not critical to the structural conclusion to be made (*vide infra*).

The relationships of (C-5)–(C-6) and (C-15)–(C-16) were distinguished from each other by comparing their chemical shifts with a structurally related model compound. Thus, the chemical shift of C-4 (41.9 ppm) of *cis*-3,5-dimethyltetrahydropyran[4] required the signal at 38.6 ppm to be assigned to C-15 and, therefore, one at 40.7 ppm to C-16. (It should be noted that the two methyls at C-14 and C-16 are *cis* and equatorially oriented.) By elimination, C-5 and C-6 were assigned to 26.4 and 28.0 ppm, respectively.

With the assignment of C-5 and C-6 thus established, it has become possible to determine the position of the methyl substituent in ring A. Comparison of the ^{13}C-nmr spectra of **I** and **II** implied the structural similarity between them except for minor modification accompanied by the following spectral differences:

(i) A new methyl signal appeared at 18.9 ppm in **I**.

(ii) A methylene (20.1 ppm) in **II** changed to a methine (28.9 ppm) in **I** shifting downfield by 8.8 ppm.

(iii) C-5-Methylene (26.4 ppm) in **II** shifted downfield by 9.1 ppm (35.5 ppm) in **I**.

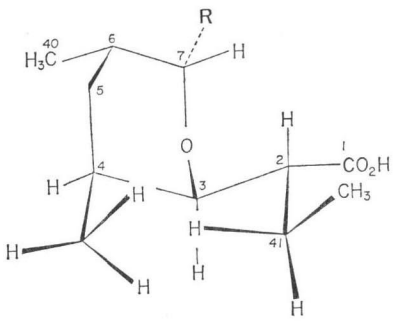
(iv) A *sec*-carbinyl carbon (74.9 ppm) in **II** moved to 78.2 ppm in **I**.

(v) No chemical shift change was observed with C-6 [28.0 ppm in **II**, 27.9 ppm in **I**].

(vi) A methyl (11.2 ppm) in **II** shifted to 12.1 ppm in **I**. Therefore, this methyl group should be assigned to C-40 due to its proximity to the methyl substituent.

In considering the chemical shift changes caused by a methyl substituent on a cyclohexane ring[5] ($\alpha_{eq}+5.7$, $\alpha_{ax}+2.0$, $\beta_{eq}+8.7$, $\beta_{ax}+5.0$, $\gamma_{ax}-5.0$, γ_{eq} and δ negligible) as well as on a tetrahydropyran ring [in case of methyl substitution at C-3, $\alpha_{eq}+3.6$, β_{eq} (to C-4)$+8.4$, β_{eq} (to C-2)$+4.4$]*, the above changes in chemical shift can best be explained by introducing the methyl substituent at C-4 but not at C-5. This conclusion established the assignment of C-4 (20.1 ppm) and C-3(74.9 ppm) in **II**. If the substitution were at C-5, C-6 would move considerably to a lower field and C-3 would remain almost unchanged.

Fig. 3. The stereochemical relationship of ring A and its substituents.



The equatorial orientation of CH_3 at C-4 was supported by the small downfield shift (+0.9 ppm) of C-40. This value can be compared with δ effect[7] reported for gauche-*trans* orientation [CH_3(C-4), equatorial]. However, since caution must be exercised in the use of δ-effect in the assignment of stereochemistry[8], the above conclusion was further corroborated by measurement of T_1 of **I**. The relaxation time of the new methyl group (T_1 0.09 sec) can be compared with methylenes (T_1 0.05~0.12 sec) and is in marked contrast to those of the remaining methyl groups (T_1 C-33: 0.22, C-40: 0.58, other methyls 0.31~0.65 sec). This unusually short relaxation time of CH_3(C-4) is explained by an energetically preferred conformation shown in Fig. 3, in which free rotation of the methyl group is prevented by a proton of C-41. Such spatial relationship is possible only when the methyl is equatorially oriented at C-4.

Fig. 3 also explains the lack of the γ effect on C-2 by CH_3(C-4) [48.9 in **II**, 49.2 ppm in **I**]. The hydrogen at C-2 is displaced not to suffer 1,3-diaxial interactions by CH_3(C-4).

Thus, the absolute stereostructure of 4-methylsalinomycin (narasin) has been established

* This effect was calculated by comparing tetrahydropyran[6] with *cis*-3,5-dimethyltetrahydropyran[4], and by assuming the γ_{eq} effect being negligible as observed in case of cyclohexane.[5]

as shown in Fig. 1*.

More recently, DORMAN *et al*[9] have reported the assignment of ^{13}C-nmr spectrum of narasin. However, it is not of use to establish the structure of **I**, since they used their biosynthetic result to help assign the spectrum of **I** and *vice versa*, and the abnormality in T_1 of CH_3(C-4) remained to be explained.

Acknowledgement

We thank the Central Research Division, Takeda Chemical Industries, Ltd. for a sample of C-7819B and Dr. D. R. BRANNON of Eli Lilly and Company for a sample of narasin.

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(Received March 4, 1977)

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* The similarity of the CD spectra of **I** and **II** has been reported.[2]