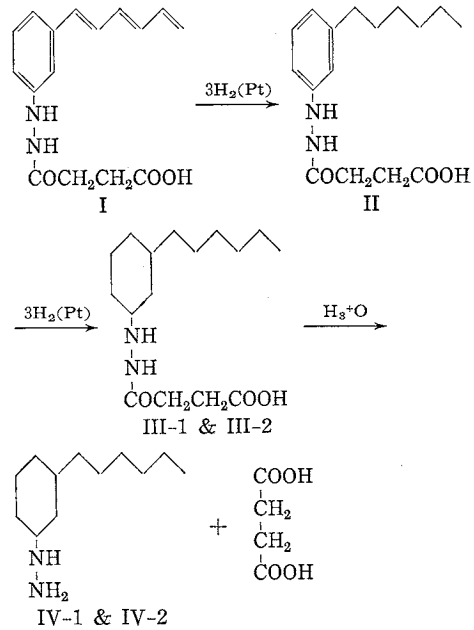
THE STRUCTURE OF SPINAMYCIN

Sir:

Spinamycin (I) is an antifungal antibiotic discovered by WANG *et al.*[1] We wish to present evidence supporting the assignment of structure I to spinamycin.

Spinamycin has the molecular formula $C_{16}H_{18}N_2O_3$*. It melts at 143°C. It has no optical activity. The ultraviolet absorption spectrum of I in methanol solution shows a maximum at 309 mμ (log ε 4.70). Essentially the same spectra are obtained in 0.1 N sodium hydroxide and 0.1 N hydrochloric acid solutions. Potentiometric titration of I in 50% aqueous methanol shows the presence of one acidic group of pKa 5.5. The presence of a free carboxyl group is suggested by the infrared absorption at 1725 cm^{-1} (KBr) which shifts to 1555 cm^{-1} by alkaline treatment. The n.m.r. spectrum** of I in hexadeuterodimethylsulfoxide solution shows one proton at δ 9.7 (singlet; COOH), about ten protons at δ 6.2~7.3, two protons at δ 5.0~5.5 and four protons at δ 2.48 (singlet). On catalytic hydrogenation in methanol solution under atmospheric pressure using platinum oxide as a catalyst, I absorbs three moles of hydrogen in 30 minutes and gradually absorbs further three moles in 20 hours.

Hexahydrospinamycin (II) is crystallized from chloroform and *n*-hexane. It melts at 101°C. It has the molecular formula $C_{16}H_{24}N_2O_3$ (M. W. 292); C 65.71, H 8.22, N 9.68, O 16.60, (calcd. C 65.72, H 8.27, N 9.58, O 16.42). It also has one carboxylic acid function of pKa 5.3 (50% aqueous methyl cellosolve solution, equivalent 290). The ultraviolet absorption spectrum of II in aqueous methanol solution shows two absorption maxima at 238 mμ (log ε 4.13) and 285 mμ (log ε 3.36). The spectrum remains unchanged in acidic and alkaline solutions. The n.m.r. spectrum of II in deuterochloroform shows three protons at δ 8.2~9.5 (broad), one proton at δ 7.0 (triplet), three protons at δ 6.5 (multiplet), six protons at δ 2.5 (multiplet), eight protons at δ 1.3 (multiplet) and three protons at δ 0.85 (triplet-like). The last two signals are the characteristic pattern of a straight (unbranched) alkyl chain which is not observed in the spectrum of I. From the above-mentioned facts, it can be speculated that spinamycin has a hexatrienyl function conjugated with an chromophoric group and hexahydrospinamycin has the chromophore linked to an *n*-hexyl group. The mass spectrum of II shows major peaks at m/e 292, 274, 204, 192, 122, 106, 91, 78, 65 and 55. The strong peaks at m/e 91, 78 and 65 suggest that II has a benzyl group as a partial structure.



Two dodecahydro-derivatives of I, being detected by thin layer chromatography, are isolated by silica gel chromatography using chloroform-ethyl acetate (1 : 1) as a developing solvent. A component III-1 is crystallized from chloroform and *n*-hexane. It melts at 97°C. Another component III-2

* In the previous report[1], $C_{16}H_{16}N_2O_2 \cdot H_2O$ was given for the molecular formula of spinamycin. The structure, presented in this report, suggests that spinamycin is easily dehydrated by heating to give a succinimide derivative. In the mass spectrum of I there was no parent peak (m/e 286). Instead, the distinct peak at m/e 268 (M-18) was observed.

** N.m.r. spectra were observed on a Varian A-60 spetrometer using tetramethylsilane as an internal reference.

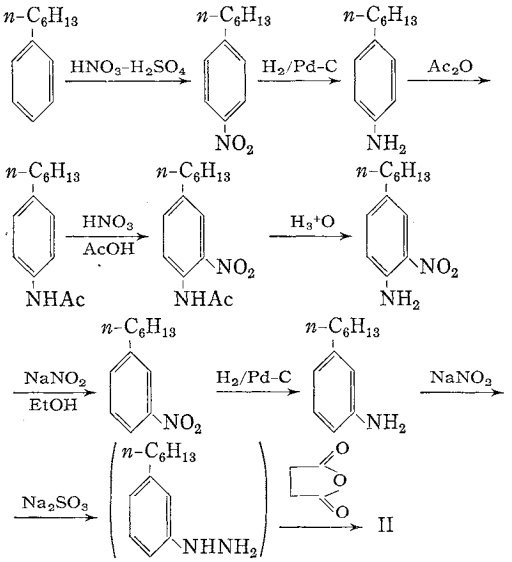
is also crystallized from chloroform and *n*-hexane. It melts at 81°C. Both have the same molecular formula $C_{16}H_{30}N_2O_3$ (M. W. 298); III-1, C 64.80, H 10.09, N 9.20, O 16.19; III-2, C 64.59, H 9.94, N 9.10, O 15.61 (calcd. C 64.39, H 10.13, N 9.39, O 16.08). They have no ultraviolet absorption except end absorption. Potentiometric titrations (in 50 % MCS) show that both have one carboxylic acid group (pKa 5.5) and one newly formed basic function (pKa 3.5). They have been confirmed to be the diasteroisomers which are formed by catalytic hydrogenation of the di-substituded benzene II.

Acid hydrolysis of III-1 gives succinic acid and a basic compound IV-1. The former is also obtained by acid hydrolysis of I, II and III-2. The basic compound is crystallized from carbon tetrachloride and *n*-hexane as the monohydrochloride, m. p. 96°C. It has the molecular formula $C_{12}H_{26}N_2 \cdot HCl$: C 61.83, H 11.51, N 11.68, Cl 15.31 (calcd. C 61.38, H 11.59, N 11.93, Cl 15.10). It has one basic group (pKa 7.5 in 50 % MCS). Positive TOLLENS and TTC (2,3,5-triphenyltetazolium chloride) reactions, together with pK studies, suggest the presence of a hydrazine group in IV-1. From acid hydrolysate of III-2, a hydrazine derivative IV-2, having the same molecular formula but giving different infrared spectrum from that of IV-1, is obtained, which has been confirmed to be the diastereoisomer of IV-1.

All of the above-mentioned facts suggest that I and II are derivatives of succinic acid phenylhydrazide and III-1 and III-2 are derivatives of succinic acid cyclohexylhydrazide.

The signals of the aromatic protons in the n.m.r. spectrum of II appear at δ 7.0 (1 H, triplet, J=7.5 cps) and δ 6.5 (3 H, multiplet). This splitting pattern suggests that the *n*-hexyl substituent of II is at meta-position to the hydrazide group.

Hexahydrospinamycin was synthesized by the following scheme:



The synthesized II was completely identical with the natural material by comparison of their infrared and mass spectra and mixed melting point.

A strong absorption band at 972 cm^{-1} of the infrared spectrum of I suggests that the hexatrienyl group of I might be all-*trans* form[2].

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References

1) WANG, E. L.; M. HAMADA, Y. OKAMI & H. UMEZAWA: A new antibiotic, spinamycin. J. Antibiotics, Ser. A 19: 216~221, 1966.
2) BELLAMY, L. J.: The infra-red spectra of complex molecules. p. 34 Methuen & Co. Ltd., London, 1958.