CH_3OH), mμ (ε): 261 (32,200), 298 (20,900), 360 (6600), 437 (18,100); M^+ 381; R_f[5] 0.73. Attempted crystallization of crude **3** by dissolving in NaOH–methanol and precipitating by acidification with HCl yielded instead the crystalline methyl ether **4**: mp 255–300° dec; $C_{21}H_{17}NO_7$; uv max (0.1 *N* HCl–CH_3OH) mμ (ε): 262 (30,400), 298 (20,200), 360 (7070), 441 (15,300); M^+ 395; R_f[5] 0.94. Both **3** and **4** were reduced in 80–90% yield to 6-methylpretetramid (**5**) in refluxing hydriodic acid–phenol.

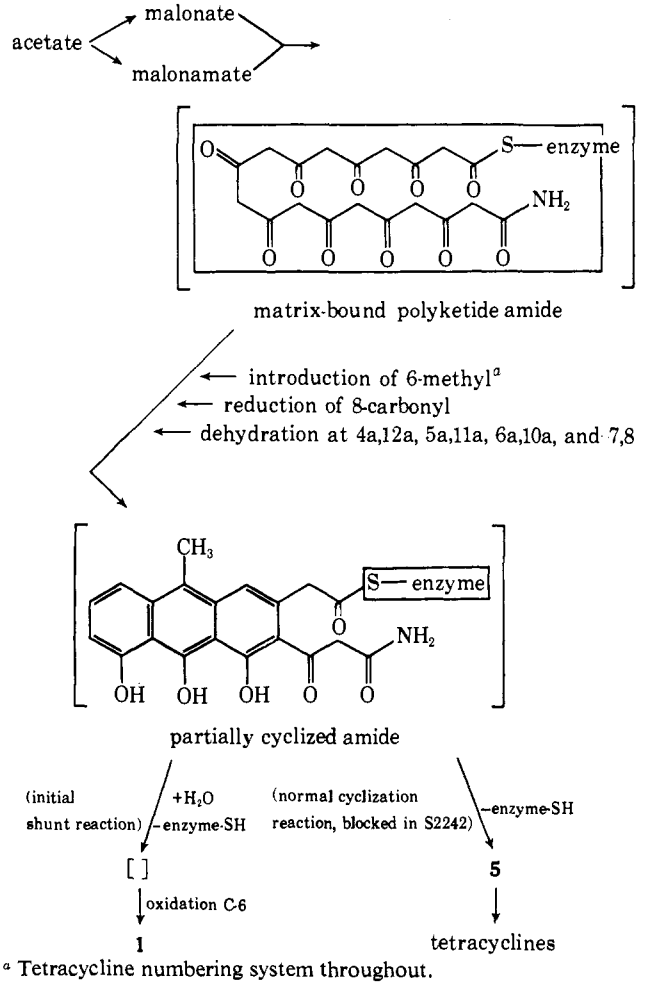
Acetylation of **1** in acetic anhydride–pyridine yielded only an amorphous acetate of the cyclization product which upon solvolysis in 0.1 *N* HCl–CH_3OH (spectrophotometric solution) yielded the methyl ether **4** of the cyclization product in 64.4% yield (spectrophotometric; theory for the pentacetate of **3**: 66.8%).

The absorption spectra and solubility properties of **3** and **4** were very similar to those of 6-methylpretetramid, but both were inert in the biological system in which 6-methylpretetramid is efficiently converted to chlortetracycline.[8]

Although **1** has an asymmetric carbon, the compound was found to be racemic, suggesting that the 9-hydroxyl is introduced chemically, by autoxidation, rather than by enzymatic hydroxylation. This, together with the observation that **1** itself is not biologically converted to tetracycline antibiotics, indicated that **1** is not an intermediate in the biosynthetic pathway but is, like protetrone, a shunt product from that pathway. The structures of **1** and protetrone show that the 6-methyl of the tetracycline molecule is introduced before the cyclization of the naphthacene system is completed, in confirmation of our conclusion based on pretetramid conversion data.[8] Likewise, we can now say with certainty that the carboxamide function is generated before cyclization is completed, a conclusion not contrary to Gatenbeck's hypothesis[9] that the carboxamide group—as malonamic acid or its biological equivalent—is the starting point in the biosynthesis of the tetracyclines. Similarly we can say that the missing oxygen at C-8 of the tetracycline molecule ("missing" in the sense that the hypothetical polyketide intermediate would have a carbonyl oxygen at that point, and removal is not obviously essential in the course of later reactions) is also removed before the cyclization to a naphthacene.

The early stages of the biosynthetic pathway to the tetracyclines can now be delineated as in Scheme I.[10]

Scheme I. Early Stages of the Biosynthesis of the Tetracyclines



[a] Tetracycline numbering system throughout.

(8) J. R. D. McCormick, S. Johnson, and N. O. Sjolander, *J. Am. Chem. Soc.*, **85**, 1692 (1963).

(9) S. Gatenbeck, *Biochem. Biophys. Res. Commun.*, **6**, 422 (1961).

(10) J. R. D. McCormick in "Antibiotics," Vol. II, D. Gottlieb and P. D. Shaw, Ed., Springer-Verlag, New York, N. Y., 1967.

J. R. D. McCormick, Elmer R. Jensen, Nancy H. Arnold Howard S. Corey, Ursula H. Joachim, Sylvia Johnson Philip A. Miller, Newell O. Sjolander

Lederle Laboratories, American Cyanamid Company Pearl River, New York 10965

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Evernitrose, a Naturally Occurring Nitro Sugar from Everninomicins[1]

Sir:

We wish to present our evidence for the structure and stereochemistry of evernitrose, the first naturally occurring nitro sugar to be isolated. Evernitrose was obtained from everninomicins B and D[2] on hydrolysis with aqueous acid, followed by chromatography of the resulting product mixture on silica gel.

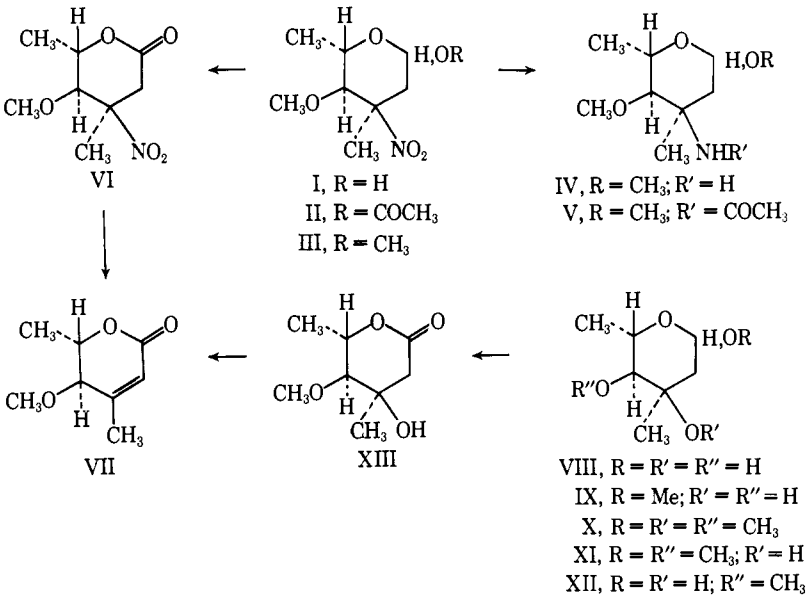
Evernitrose (I, $C_8H_{15}NO_5$;[3] mp 88–93°; $[\alpha]_D$ −4.9° → −19.4° (EtOH, 24 hr); λ_{max}^{MeOH} 283 mμ (ε 52.5); λ_{max}^{Nujol} 2.99, 6.44 μ (nitro)) formed a monoacetate (II, $C_{10}H_{17}NO_6$; mp 58–59°, $[\alpha]_D$ −20.5° (EtOH); λ_{max}^{Nujol} 5.68 (acetate), 6.44 μ, no hydroxyl absorption). The nmr spectrum of the acetate showed the presence of a secondary methyl (δ 1.38; J = 7 cps), a tertiary methyl (δ 1.71), an acetate methyl (δ 1.95), a methoxyl (δ 3.88), a one-proton multiplet at δ 3.55, a one-proton doublet at δ 3.38 (J = 6 cps), and a one-proton quartet at δ 5.80 for the axial anomeric proton (J_{aa} = 8 cps; J_{ae} = 3 cps).

The presence of the nitro group in I was indicated by an M − NO_2 (m/e 159) peak in the mass spectrum and by its ir and uv spectra. Further confirmation was

(1) The Chemistry of Everninomicin Antibiotics. III. Reference 2a may be considered as part I. Part II: H. Reimann, R. S. Jaret, and O. Z. Sarre, to be published.

(2) (a) H. L. Herzog, E. Meseck, S. DeLorenzo, A. Murawski, W. Charney, and J. P. Rosselet, *Appl. Microbiol.*, **13**, 515 (1965); (b) M. Weinstein, G. M. Luedemann, E. M. Oden, and G. H. Wagman, "Antimicrobial Agents and Chemotherapy—1964," American Society for Microbiology, Ann Arbor, Mich., 1965, p 24.

(3) Satisfactory elementary analyses were obtained for all new compounds; ir spectra were recorded in chloroform solution unless otherwise noted; nmr spectra were taken at 60 Mc in $CDCl_3$ with internal TMS standard; optical rotations were measured in chloroform solution at 25°, unless otherwise noted.



obtained chemically as follows. Evernitrose methyl glycoside (III), prepared by allowing evernitrose to react with methanolic hydrogen chloride, was catalytically hydrogenated[4] using 10% palladium on charcoal, to afford the amine IV, λ_{max} 3 μ (amino), no absorption for the nitro group. The amine was characterized as its acetyl derivative, V, $C_{11}H_{21}NO_4$; mp 114–115°, $[\alpha]D$ −30°; λ_{max} 3.0 (NH), 5.94 μ (amide). The nmr spectrum showed the presence of an acetamido methyl signal at δ 1.95 which is suggestive of the axial configuration of the acetamido group in V. It has been predicted[5] that the expected range for axial acetamido groups on carbon bearing methyl would be δ 1.93–1.86, compared to δ 1.87–1.78 for their equatorial counterparts. In the absence of any suitable example of an axially oriented acetamido group on a fully substituted carbon atom our assignment of configuration at C_3 is tentative.

Evernitrose (I) on oxidation with bromine water yielded a δ lactone (VI), $C_8H_{13}NO_5$; mp 63–64°, $[\alpha]D$ −70°; λ_{max} 5.69 (δ lactone), 6.44 μ (nitro), no hydroxyl absorption. The nmr spectrum of VI showed an AB pair of doublets centered at δ 3.10 (J = 18 cps), a secondary methyl at δ 1.50 (J = 6.5 cps), a tertiary methyl at δ 1.70, a methoxyl at δ 3.51, a one-proton doublet at δ 3.83 (J = 9 cps), and a one-proton octet at about δ 4.2. When refluxed with methanolic potassium acetate, δ lactone VI was smoothly converted to a colorless liquid (VII), $C_8H_{12}O_3$, sublimed at 40° (0.1 mm); $[\alpha]D$ −38.6°; $\lambda_{max}^{cyclohexane}$ 205 mμ (ϵ 11,200); λ_{max} 5.75 μ (α,β-unsaturated δ lactone), no absorption for the nitro group. The nmr spectrum showed the presence of a vinyl methyl at δ 2.10, a vinyl hydrogen at δ 5.83, a one-proton doublet at δ 3.60 (J = 7 cps), a one-proton quintet at δ 4.50 (J = 7 cps), a secondary methyl at δ 1.43 (J = 7 cps), and a methoxy at δ 3.50.

The above sequences of reactions establish the structure of evernitrose and its relative stereochemistry at C_4 and C_5.

In order to prove the gross structure of evernitrose unequivocally and also to assign the absolute stereochemistry at C_4 and C_5, compound VII has been synthesized from mycarose[6] (VIII) in the following way. Mycarose methyl glycoside (IX) on methylation[6a] using sodium hydride and methyl iodide gave a mixture of the 3,4-di-*O*-methyl derivative X and the 3-*O*-methyl derivative XI which was separated on a silica gel column. Mycarose 1,4-dimethyl ether (XI) on hydrolysis with aqueous acid yielded XII, $C_8H_{16}O_4$; mp 134–135°, $[\alpha]D$ −19.3° → −81.8° (24 hr). Oxidation with bromine water yielded the δ lactone XIII, $C_8H_{14}O_4$; mp 118–119°; $[\alpha]D$ −71.5°; λ_{max} 2.80, 5.70 μ. The nmr spectrum of XIII was consistent with the assigned structure. The δ lactone XIII, when refluxed in benzene solution in the presence of a catalytic amount of *p*-toluenesulfonic acid, yielded the desired α,β-unsaturated δ lactone VII, $[\alpha]D$ −37.5°, which was identical (ir, uv, nmr, tlc) with the sample of the α,β-unsaturated lactone obtained from I. The synthesis of VII from mycarose (VIII) proves the gross structure of evernitrose (I) and also its absolute stereochemistry at C_4 and C_5 as 4-*O*-methyl-3-*C*-methyl-3-nitro-2,3,6-trideoxy-L-*ribo*- (or -*arabino*) hexose.

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A. K. Ganguly, Olga Z. Sarre, Hans Reimann
Natural Products Research Department
Schering Corporation, Bloomfield, New Jersey 07003
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Photochemical Interconversion of Cyclooctatetraene Bond Shift Isomers[1]

Sir:

The rates of bond shift in cyclooctatetraene and a number of its derivatives have been determined by nuclear magnetic resonance spectroscopy.[2] In all the

(1) This work was supported by the National Science Foundation, Grant No. G. P. 6620.

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