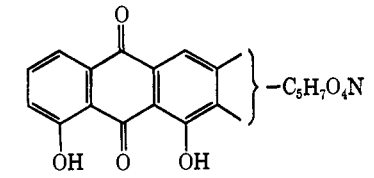
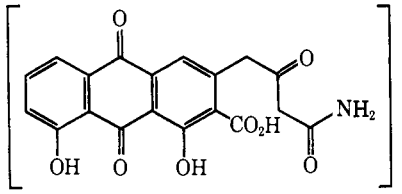
2. This product was also obtained in high yield by the dehydration of **1** in HBr–acetic acid: **2**, mp >300°; $C_{19}H_{11}NO_7 \cdot H_2O$; λ_{max} (H_2SO_4–H_3BO_3) mμ (ε): 280 (15,300), 297 (63,600), 418 (9600), 508 shoulder (8400), 553 (17,500), 593 (22,000). Refluxing **1** in 58% HI–phenol gave a moderate yield (30% of theory) of pretetramid (**3**), identified by the characteristic absorption spectra of the crystalline product.[8]

The visible-uv absorption spectrum of **1** and the dehydration to **2** established that the partial structure of protetrone was **6** and this, together with further biogenetic considerations, suggested the possible structures for protetrone, **7** or **1**. The choice between these was made possible by the observation that, on standing



6



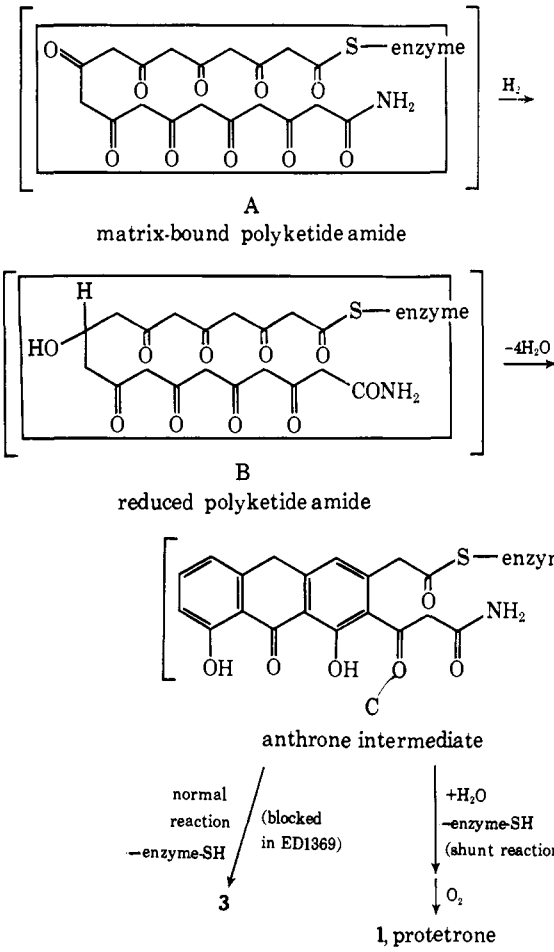
7

at room temperature for 8 days in DMSO solution, protetrone lost 1 mol of CO_2[9] to yield an anthraquinone product, **4**: mp 190–200° dec $C_{18}H_{13}NO_6$; λ_{max} (0.1 *N* HCl–methanol) mμ (ε): 256 (26,200), 287 sh (12,100), 431 (11,450); δ_{TMS} (DMSO), ppm: 2.3 and 2.4 (aryl methyl, split by keto–enol tautomer), 3.8 (methylene of β-keto amide), 5.3 (vinyl proton of enol), 7.1–7.9 (complex of aryl and amide), 12.2 (enol). Nmr spectra showed **4** to be a tautomeric keto–enol mixture having an aryl methyl group. Thermal degradation of **4** in refluxing DMSO resulted in the further loss of the carboxamide group (possibly as HOCN) to yield the anthraquinone, **5**: mp 197–199° dec; $C_{17}H_{12}O_5$; λ_{max} (0.1 *N* HCl–methanol) mμ (ε) 226 (32,200), 255 (23,200), 278 sh (11,400), 288 (11,000), 430 (11,550); ir absorption max, cm^{-1}: 1700 (hindered $ArCOCH_3$), 1670 (quinone C=O); δ_{TMS} ($DMSO$–$MgHCOO)_2$, ppm: 2.20 (acetyl), 2.5 (aryl methyl), 7.0–8.0 (complex of aryl protons), now having aryl methyl and acetyl methyl proton resonances in the nmr spectrum. These properties are consistent only with structure **1**.

Protetrone has shown no biological activity as a tetracyclines precursor, indicating that it is probably not an intermediate. The close relationship to pretetramid, and therefore to the tetracyclines, however, suggests that **1** is a shunt product arising by oxidation of the corresponding anthrone C, which in turn is available because of the presence of a block in ED1369 for the final cyclization reaction leading to pretetramid (Scheme I).

Scheme I. Postulated Origin of Protetrone[10]

malonate + malonamate ⟶



The biogenetic significance of the structure of protetrone is discussed in detail in the accompanying communication.[11] The discovery of this incompletely cyclized polyketide renews hope that still earlier intermediates, or structurally significant shunt products of these, may be stable enough to accumulate in blocked-mutant fermentations.

(8) J. R. D. McCormick, J. Reichenthal, S. Johnson, and N. O. Sjolander, *J. Am. Chem. Soc.*, **85**, 1694 (1963).

(9) This extremely facile decarboxylation of a phenylacetic acid derivative appears to be characteristic of the compound and may involve internal assistance by the neighboring malonamoyl substituent. Decarboxylation apparently involves the anion as it is prevented by acidification of the DMSO solution. Homophthalic acid does not undergo thermal decarboxylation under these or even more drastic (reflux in DMSO) conditions.

(10) J. R. D. McCormick in "Antibiotics," Vol. II, D. Gottlieb and P. D. Shaw, Ed., Springer-Verlag, New York, N. Y., 1967, pp 113–122.

(11) J. R. D. McCormick, E. R. Jensen, N. H. Arnold, H. S. Corey, U. H. Joachim, S. Johnson, P. A. Miller, and N. O. Sjolander, *J. Am. Chem. Soc.*, **90**, 7127 (1968).

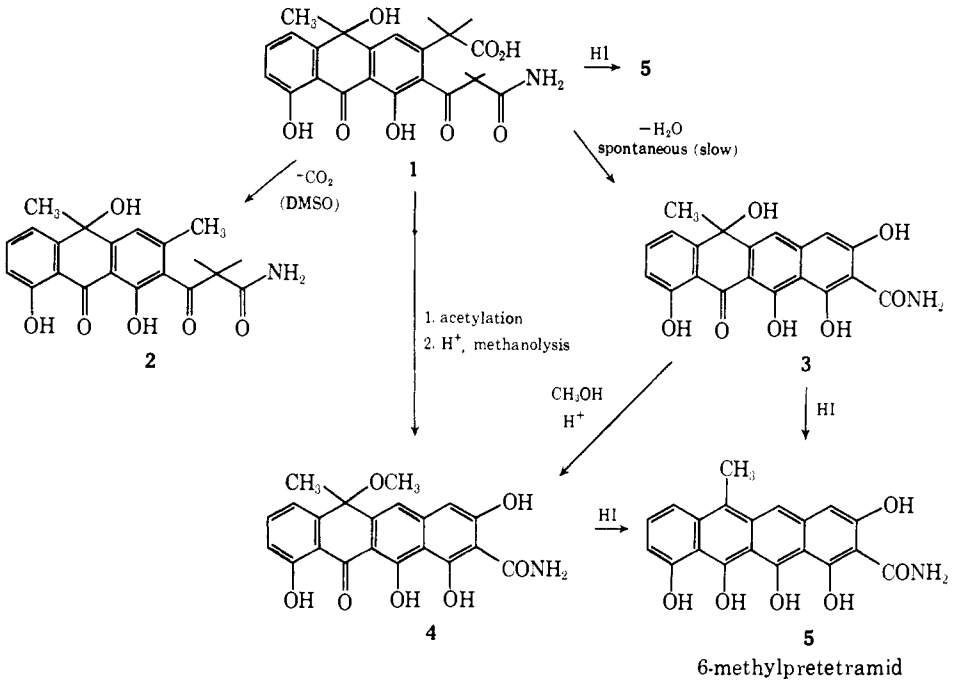
J. R. D. McCormick, Elmer R. Jensen
Lederle Laboratories, American Cyanamid Company
Pearl River, New York 10965
Received September 3, 1968

Biosynthesis of Tetracyclines. XI[1]. The Methylanthrone Analog of Protetrone

Sir:

In an accompanying communication[1] we have described the isolation and structure determination of protetrone, an anthraquinone shunt product from the biosynthetic pathway to the 6-demethyltetracyclines.

(1) Previous paper in this series: J. R. D. McCormick and E. R. Jensen, *J. Am. Chem. Soc.*, **90**, 7126 (1968).



We have now isolated the analogous methylanthrone, **1**, from a *Streptomyces aureofaciens* mutant, S2242. This mutant of a chlortetracycline-producing parental strain has been known to us for some years.[2] During that time we have made several attempts to determine whether it accumulated tetracycline-related metabolites. The discovery of mutant V828 as a spontaneous variant of S2242 increased our interest in the latter when we determined that V828 was indistinguishable from ED1369, the mutant from which we isolated protetrone. ED1369 evidently had been derived from its methylation-blocked parent by introduction of a second block at the same site as that in S2242; V828 in turn was derived from the methylating S2242 by the addition of a block for methylation. Since S2242 is visibly different from the other two, we concluded that the site for methylation must precede the point at which the three mutants have their common, primary block (this follows from the presumption that intermediates leading to the methylated and nonmethylated tetracyclines are identical up to the point of methylation). To test this conclusion, we engaged in the task of isolating from S2242 the methylated metabolite which would correspond to protetrone. The isolation of 9,10-dihydro-3-malonamoyl-9-methyl-10-oxo-4,5,9-trihydroxy-2-anthraceneacetic acid (**1**) was finally accomplished by filtration of the mash at pH 6.7, extraction from the filtrate into methyl isobutyl ketone at pH 3, partition column chromatography on diatomaceous earth (pH 10 sodium borate buffer–butanol), inspection of the multitude of partially separated components to discover the proper anthrone-like product, and crystallization of the crude **1** from acetone–ammonia–methanol as the ammonium salt monohydrate:[3] mp dec 130–250°; $C_{20}H_{22}N_2O_9$; uv max (0.1 *N* HCl–CH_3OH), mμ (ε): 250 (14,600), 260 (14,300), 270 (11,800), 300 (10,100), 372 (11,600); nmr (DCl in DMSO, *vs.* TMS) δ (ppm) 1.52 (aliphatic CH_3), 3.75 (benzylic CH_2), 6.8–7.8 (complex of aryl H and $CONH_2$); ir (KBr) 1670 cm^{-1} (C=O); R_f[4] 0.01, R_f[5] 0.65.

The ultraviolet absorption spectrum of **1** was characteristic and very similar to those of 10,10-disubstituted anthrones which we had encountered earlier.[6] The expected cyclization and reduction to 6-methylpretetramid (**5**) occurred smoothly in refluxing hydriodic acid–phenol. The nmr spectrum of the ammonium salt of **1** in DMSO unexpectedly showed a proton resonance (2.33 ppm) characteristic of an aryl methyl group as well as that of the expected isolated aliphatic methyl (1.52 ppm). Having previously observed, however, the remarkably facile decarboxylation of protetrone in aprotic polar solvents,[7] we considered the possibility that this also was happening to **1** in the nmr solution. When the nmr examination was repeated in DMSO which was acidified[7] with DCl, the expected benzylic proton resonance was seen at 3.75 ppm as indicated above, and the aryl methyl resonance was not present. Paper chromatographic examination of DMSO solutions of the ammonium salt of **1** and of the corresponding free acid confirmed the extreme instability of the salt in DMSO, yielding in a few minutes at 25° the decarboxylated product **2** (not isolated in pure form): uv max (0.1 *N* HCl–CH_3OH) mμ (ε, approximate): 255 (13,000), 269 sh (11,000), 301 (9800), 371 (11,000); nmr (DMSO *vs.* TMS) δ ppm 1.52 (aliphatic CH_3), 2.33 (aryl CH_3), 6.8–7.8 (complex of aryl H and $CONH_2$); M^+ 355; R_f[4] 0.81.

Long storage at 25° of a specimen of the amorphous sodium salt of **1** resulted in partial cyclization to yield **3** (crystallized from dimethylformamide–0.1 *N* HCl); mp >250° dec; $C_{20}H_{15}NO_7$; uv max (0.1 *N* HCl–

(2) J. R. D. McCormick, U. Hirsch, N. O. Sjolander, and A. P. Doerschuk, *J. Am. Chem. Soc.*, 82, 5006 (1960).

(3) Satisfactory microanalyses were obtained for all compounds where an elemental formula is indicated.

(4) The chromatographic system was benzene–acetone–water (2:1:2) as described by L. L. Smith, T. Foell, R. DeMaio, and M. Halwer, *J. Am. Pharm. Assoc., Sci. Ed.*, 48, 528 (1959).

(5) The chromatographic system was 1-butanol–0.1 M EDTA (pH 8.3). Whatman No. 1 paper was dipped in the aqueous phase and dried before use. The butanol phase was used to develop the strips in the descending mode.

(6) J. R. D. McCormick and E. R. Jensen, *J. Am. Chem. Soc.*, 87, 1794 (1965).

(7) See Footnote 9 in ref 1.

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.

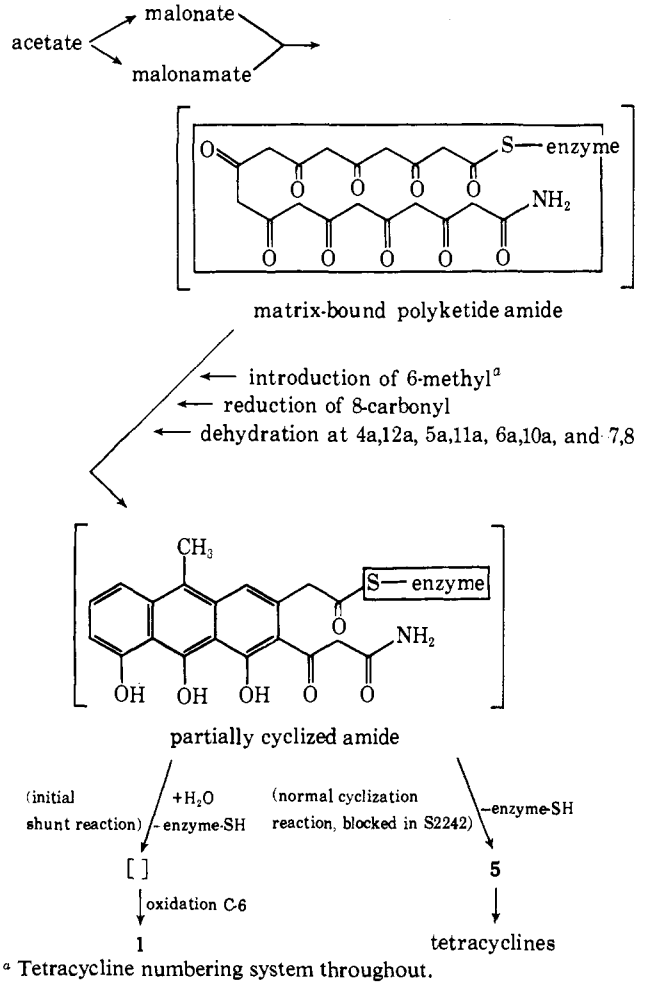
Acetylation of **1** in acetic anhydride–pyridine yielded only an amorphous acetate of the cyclization product which upon solvolysis in 0.1 *N* HCl–CH_3OH (spectrophotometric solution) yielded the methyl ether **4** of the cyclization product in 64.4% yield (spectrophotometric; theory for the pentacetate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