gies of the two transition states varies from 4.2 kcal/mol for the parent tertiary system, down to 1.6 kcal/mol for 2,7,7-trimethyl-2-norbornyl, and up to 7.1 kcal/mol for 2,6,6-trimethyl-2-norbornyl.

These results clearly establish the importance of steric effects as a factor in the *exo*:*endo* rate and product ratios in the norbornyl system.

Acknowledgment. We are deeply indebted to Professor Paul von R. Schleyer of Princeton University for relinquishing his own plans for a closely related study when he learned of our interest in the problem. He generously supplied us with a sample of 6,6-dimethyl-2-norbornanone, which facilitated the initial experiments.

(9) Postdoctorate research associate on a grant (GP 6492 X) supported by the National Science Foundation.
(10) National Science Foundation Cooperative Fellow, 1965–1967.

Shiro Ikegami,[9] David L. Vander Jagt,[10] Herbert C. Brown
Richard B. Wetherill Laboratory
Purdue University, Lafayette, Indiana 47907
Received August 22, 1968

Biosynthesis of Tetracyclines. X. Protetrone[1]

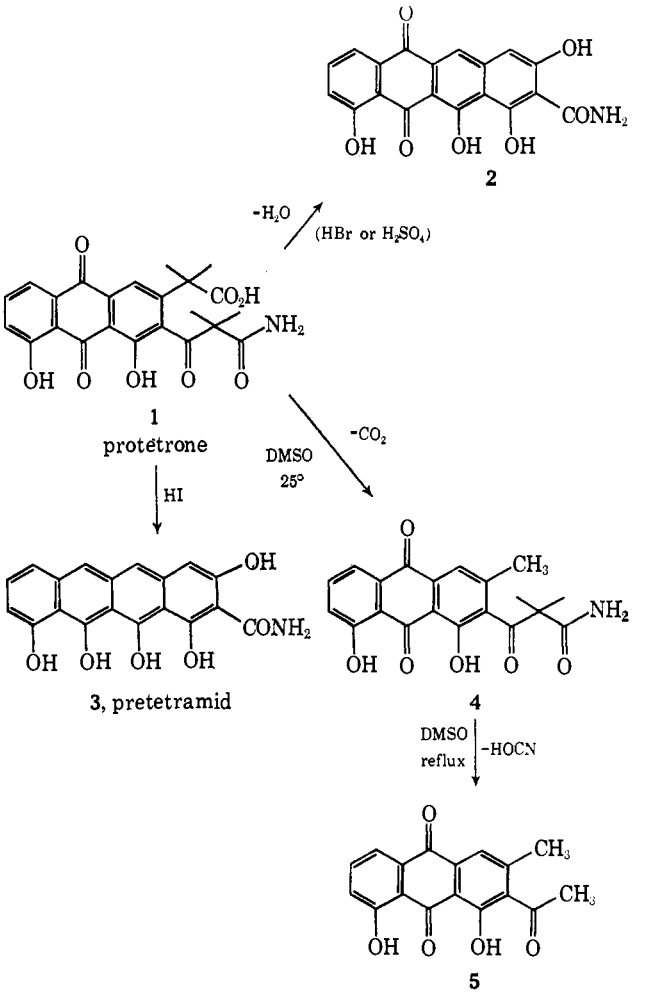
Sir:

A type of mutant frequently encountered in working with the demethyltetracycline-producing strains of *Streptomyces aureofaciens* is characterized by the development of rust-colored pigmentation in colonies on agar plates. These mutants are often low or nonproducers of antibiotic activity but are active in cosynthetic systems with other point-blocked mutants and are efficient converters of biosynthetic intermediates to produce antibiotic activity. One such isolate,[2] ED-1369, is typical of the group. Mutant ED1369 produces less than 1 mμg/ml of antibacterial activity (as demethylchlortetracycline). It is effective in converting to the corresponding antibiotic each of the known tetracycline biosynthetic intermediates, and it shows in mixed fermentations a cosynthetic response[3] with all other noncoincident point-blocked *S. aureofaciens* mutants. The product of each ED1369 cosynthetic system is the particular antibiotic which is characteristic of the other member of the system; therefore we have come to consider ED1369 to be the "universal acceptor" mutant and have long assumed it to be point blocked at a relatively early site in the biosynthetic pathway to the tetracyclines. As pretetramid is among the biologically convertible intermediates,[4] it is evident that ED1369 must be blocked at a point preceding that at which the pretetramids appear.

We wished to determine whether, among the metabolic products of ED1369, there might be substances recognizably related to the tetracyclines. Since much prior experience has indicated that the pigments of *S. aureofaciens* mutants are frequently tetracycline related,[5,6] an investigation of the pigments of ED1369 constituted our starting point. The shaker-flask mash of this mutant is dark maroon and the pigment was found to be almost entirely associated with the mycelial solids.

The isolation of 9,10-dihydro-4,5-dihydroxy-3-malonamoyl-9,10-dioxo-2-anthraceneacetic acid (protetrone, **1**) was accomplished by extraction into acidic tetrahydrofuran and fractional precipitation with hexane. The crude product was further purified by conversion to the sodium salt and back to the free acid, then recrystallized from acidified dimethyl sulfoxide–methylene chloride. After drying over P_2O_5 *in vacuo*, **1** was obtained as an orange crystalline solid:[7] mp 186–190° dec; $C_{19}H_{13}NO_8$; λ_{max} (0.1 *N* HCl–methanol) mμ (ε) 255 (25,200), 276 sh (14,200), 286 sh (11,750), 432 (11,750); ir absorption max, cm^{-1}: 1700 (CO_2H), 1670 (quinone C=O), 1630 (amide C=O); δ_{TMS} (DMSO), ppm: 3.80 (benzylic methylene), 3.89 (methylene of β-keto amide), 5.28 (enol vinyl), 7.0–8.0 (complex of amide and aryl), 11.70 (carboxyl), 12.32 (enol hydroxyl).

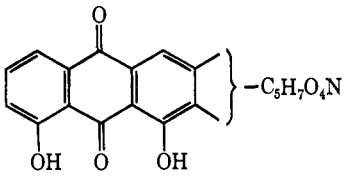
Zinc dust distillation of **1** yielded anthracene; solution in sulfuric–boric acid initially showed a characteristic absorption spectrum [λ_{max} mμ (ε): 246 (23,800), 274 (26,600), 295 (23,600), 510 (16,800), 538 (16,500)] which slowly changed (24 hr at 25°) to essentially the spectrum of the known naphthacenequinone,[5]



(1) Previous paper in this series: J. R. D. McCormick, E. R. Jensen, S. Johnson, and N. O. Sjolander, *J. Am. Chem. Soc.*, **90**, 2201 (1968).
(2) Mutant ED1369 was selected from a demethylchlortetracycline-producing parent by Mr. N. Deduck and Dr. John Growich of these laboratories.
(3) J. R. D. McCormick, U. Hirsch, N. O. Sjolander, and A. P. Doerschuk, *J. Am. Chem. Soc.*, **82**, 5006 (1960).
(4) J. R. D. McCormick, S. Johnson, and N. O. Sjolander, *ibid.*, **85**, 1692 (1963).
(5) J. R. D. McCormick and W. E. Gardner, U. S. Patent 3,074,975 (1963).
(6) J. R. D. McCormick and E. R. Jensen, *J. Am. Chem. Soc.*, **87**, 1794 (1965).
(7) Satisfactory microanalyses were obtained for all compounds where the composition is indicated.

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



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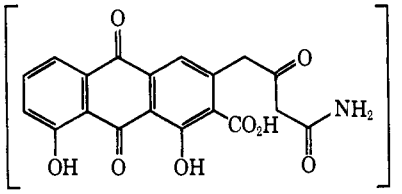

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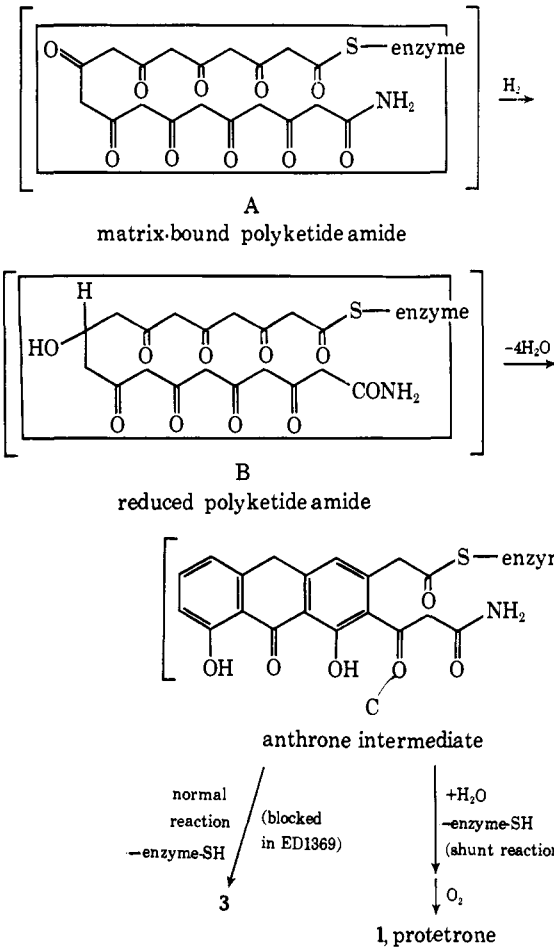
at room temperature for 8 days in DMSO solution, protetrone lost 1 mol of CO_2[9] to yield an anthraquinone product, **4**: mp 190–200° dec $C_{18}H_{13}NO_6$; λ_{max} (0.1 *N* HCl–methanol) mμ (ε): 256 (26,200), 287 sh (12,100), 431 (11,450); δ_{TMS} (DMSO), ppm: 2.3 and 2.4 (aryl methyl, split by keto–enol 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).