smaller than that obtained by titration experiments, we consider the agreement sufficiently good in view of the differences in experimental approach and experimental errors.

The almost quantitative agreement among the values of the intramolecular stacking quotient or percent stacking at 25 °C obtained by the three independent procedures gives strong substantiation of the two-state model for describing the intramolecular stacking association of ϵApϵA. This is one of the principal conclusions from the present results.

Next, in connection with literature discrepancies[2-4] with regard to the degree of stacking, the present conclusion is contrary to the recent view of Baker et al.[4] and Lee and Tinoco,[3] and is in favor of the previous conclusion drawn by Tolman et al.[2,20] (Baker et al.[4] have obtained the thermodynamic parameters for ϵApϵA from the temperature-dependent circular dichroic data in *2 M NaCl*: $\Delta H° = -29$ kJ/mol and $\Delta S° = -113$ J mol^{-1} deg^{-1}. These values indicate that the stacking interaction is weaker than ApA.) It should be noted that such a tendency for the stacking interaction to be enhanced by the chloroacetaldehyde modification reaction has been observed in the intermolecular association of ϵAdo and the intramolecular association of ϵFAD before[22] (s_0 values at 20 °C of FAD and ϵFAD are 5 and 9, respectively.[22])

Finally, comparison between intermolecular and intramolecular stacking association constants shows that the former is greater than the latter. Such a tendency has also been observed in purine and other three-ring heterocyclic systems.[23-25] The ratio of the equilibrium quotients for the intramolecular and intermolecular associations is about 0.11 M. Here, we choose 1 *m* as a standard state for comparison simply because of convenience. If the comparison were based on a mole fraction standard state, it would be about 2.2×10^{-3} mole fraction ($\Delta S_{inter} - \Delta S_{intra} \simeq 0$ J (mol fraction)$^{-1}$ deg^{-1} on a unitary standard state, and $\Delta H_{inter} - \Delta H_{intra} = -14.4$ kJ/mol). This advantage from intermolecularity seems to result from a more extensive degree of overlapping of the bases in intermolecular association than in intramolecular association. In other words, the advantage could be gained if the intermolecular geometry of ϵAdo has more overlap than the stacking geometry in ϵApϵA in aqueous solution. Thus, the observed increase in the equilibrium quotient for the intermolecular association of ϵAdo may be mainly due to increased surface tension forces (enthalpy driven-entropy opposed) compared with the intramolecular stacking in ϵApϵA. It should, however, be noted that this situation is not necessarily true for every case, but different pairs of nucleoside and dinucleoside phosphate may behave differently. Nevertheless, it has generally been noticed that the stacking equilibrium quotients for intermolecular associations are greater than those for the corresponding intramolecular associations, presumably because the linkage of the two nucleosides through the phosphodiester bond introduces new steric constraints on the possible degree of base overlap.

Other intermolecular stacking associations that show a large increase in the unitary standard free energy changes relative to their intramolecular counterparts will be reviewed elsewhere.

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(24) Kallenbach, N. R.; Berman, H. M. *Q. Rev. Biophys.* **1977**, *10*, 138-236.
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Photochemical Transformations. 28.[1] Comparisons of "Ionic" Intermediates Produced Photochemically with Corresponding Ground-State Intermediates. Further Studies in Some Chlorobenzobicyclooctadienyl Systems

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Abstract: Irradiation of *exo*-2-deuterio-6,7-benzo-*endo*-2-bicyclo[3.2.1]octadienyl methanesulfonate (**8**-OMs) and its epimer, *endo*-2-deuterio-6,7-benzo-*exo*-2-bicyclo[3.2.1]octadienyl methanesulfonate (**10**-OMs), demonstrates that an unsymmetrical species intervenes in the photorearrangements observed, while the photosolvolyses proceed via intermediates similar to those of ground-state solvolyses. Plausible rationalizations are discussed briefly.

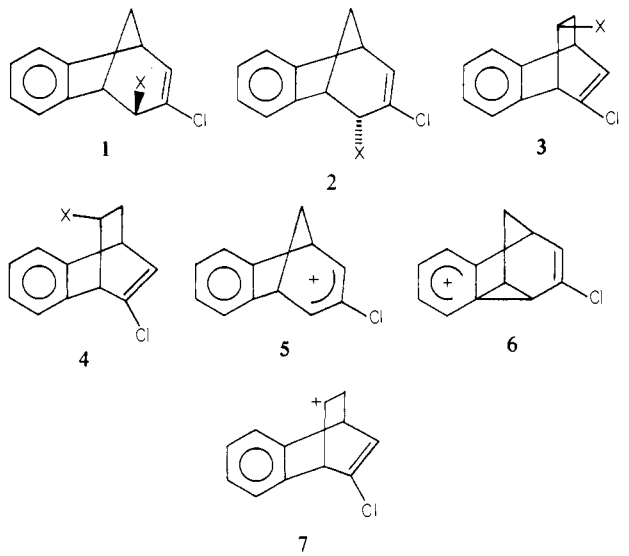
About a decade ago, members of this research group suggested[2] the intermediacy of carbocations in certain photochemical rearrangements, following the earlier reports[3] of carbocation intermediates in photosolvolysis of certain benzyl derivatives. Since that time there have been many reports[4] of carbocation formation in photochemically induced (or photosensitizer induced) rearrangements and solvolyses, so that it is now clear that bond heterolysis may be the result of photoexcitation or photosensitization in a variety of systems. Still to be determined are the kinds of mechanistic paths[6,7] leading to the carbocationic intermediates, which may, of course, differ with different substrates, different environments, and different excited-state multiplicities, and the question of whether the carbenium ions produced from the excited

(1) Part 27: Cristol, S. J.; Daussin, R.D. *J. Am. Chem. Soc.*, **1980**, *102*, 2866.
(2) Cristol, S. J.; Mayo, G. O.; Lee, G. A. *J. Am. Chem. Soc.* **1969**, *91*, 214.
(3) (a) Zimmerman, H. E.; Sandel, V. R. *J. Am. Chem. Soc.* **1963**, *85*, 915. (b) Zimmerman, H. E.; Somasakhara, S. *Ibid.* **1963**, *85*, 922.
(4) For leading references see ref 5 and 6.
(5) Cristol, S. J.; Stull, D. P.; McEntee, Jr., T. E. *J. Org. Chem.* **1978**, *43*, 1756.
(6) Cristol, S. J.; Stull, D. P.; Daussin, R. D. *J. Am. Chem. Soc.* **1978**, *100*, 6674.
(7) (a) Kropp, P. J.; Jones, T. H.; Poindexter, G. S. *J. Am. Chem. Soc.* **1973**, *95*, 5420. (b) Kropp, P. J.; Poindexter, G. S.; Pienta, N. J.; Hamilton, D. C. *Ibid.* **1976**, *98*, 8135.

0002-7863/80/1502-5577$01.00/0

states (presumably exergonic reactions) differ from those produced in ground-state solvolyses. It is the latter question that this report addresses.

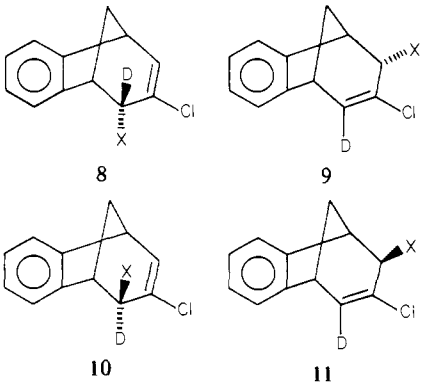
We have recently reported[8] results on the ground-state and photochemical reactions of certain derivatives represented by formulas **1**–**3**, that is, certain chlorobenzobicyclo[3.2.1]octadienyl and chlorobenzobicyclo[2.2.2]octadienyl systems. That work demonstrated that the **1**–**3** systems were readily interconverted in the ground state, with *exo*-**1** derivatives being "kinetic" products, that epimerization to exo–endo mixtures (**1**–**2**) occurs readily, that rearrangements to the anti [2.2.2] isomers **3**-X occur somewhat less readily, that the **3**-X isomers are the thermodynamically more stable ones, and that there is a high-energy mechanistic barrier separating this anti allylic system from the isomeric syn benzylic system which can be derived from the solvolysis of, for example, **4** derivatives. These results led us to suggest that, among the plausible carbocations in the anti allylic systems, the allylic ion **5** is the most stable, the bridged phenonium ion **6** is less stable, but nonetheless accessible, and the secondary cation **7** is too high in energy to have been accessible in our experiments.



We also found that direct irradiation with 254-nm light of **1** and **2** compounds with good nucleofugal groups (e.g., X = Cl, OSO_2CH_3, $OCOCHCl_2$) in acetonitrile, followed by water treatment, led to the photosolvolysis product **1**-$NHCOCH_3$ (or, in wet acetonitrile, to **1**-$NHCOCH_3$ and **1**-OH), accompanied by photoisomerization to give mixtures of **1**, **2**, and **3**. With poor nucleofugal groups (X = OAc, OH) no photo-Wagner–Meerwein rearrangements or photosolvolyses were observed, and instead di-π-methane rearrangements occurred.[9] These results are consistent with the idea that bond heterolysis was involved with the good nucleofugal groups. Detailed studies with the methanesulfonates showed that irradiation of *exo*-**1**-OMs gave **3**-OMs in a quantum yield of about 0.25 and **1**-$NHCOCH_3$ with a quantum yield of about 0.15, and with little or no *endo*-**2** methanesulfonate or amides **2** or **3** formed. The *endo*-methanesulfonate **2**-OMs gave **3**-OMs, **1**-OMs, and **1**-$NHCOCH_3$ in quantum yields approximating 0.2, 0.1, and 0.2, respectively, and with no **2**-$NHCOCH_3$ or **3**-$NHCOCH_3$ observed.

The photochemical solvolysis results were quite different from the ground-state results, where, for example, solvolysis of **1**-OMs in aqueous acetonitrile at reflux or at room temperature (half-life ca. 30 days) led to a mixture of **1**-OH and **1**-$NHCOCH_3$, without any measurable return to **3**-OMs (comparable conditions, but shorter times *with irradiation* gave 50% rearrangement to **3**-OMs). We suggested that the photochemical results could be accommodated best by the intervention of the cation **6** rather than the more stable **5**, as the first-formed species, presumably as a member of a tight ion pair $\mathbf{6}^+X^-$, produced from the excited-state progenitor, and that such ion pairs revert to both **1**-X and **3**-X isomers, or relax to form the more stable ion pairs $\mathbf{5}^+X^-$, whereupon the reactions of the latter (return to **1**-X and **2**-X or solvolysis to **1**-$NHCOCH_3$ and **1**-OH) could occur.

We now wish to report results on the photoinitiated solvolyses and rearrangements of deuterium-labeled **1**-OMs and **2**-OMs isomers in acetonitrile. The preparation of *exo*-2-deuterio-**2**-OMs (**8**-OMs), which was obtained by methanesulfonation of the alcohol prepared in turn by lithium aluminum deuteride reduction of the corresponding ketone, has been described.[8] Both ^1H NMR and ^2H NMR showed freedom from contamination with its allylic isomer **9**-OMs. Its epimer (**10**-OMs) was not so readily produced. A variety of displacement reactions involving acetate ion with **8**-OMs in polar solvents gave mixtures of **10**-OAc and **11**-OAc of such a nature that carbenium-ion intermediates were undoubtedly involved. Treatment of **8**-OMs with tetra-*n*-butylammonium acetate in dry benzene[10] gave **1**-OAc-d_1 which, while not pure, was 83% **10**-OAc and 17% **11**-OAc. Methanolysis of the acetate gave **1**-OH-d_1, and methanesulfonation gave **1**-OMs-d_1 which (^2H NMR analysis) was 83% **10**-OMs and 17% **11**-OMs.[12] Our studies with the exo isomer then are somewhat less precise than those with the endo isomer.



Irradiation of **8**-OMs in acetonitrile at room temperature with 254-nm light was carried out under conditions such that approximately 35% of the initial **2**-OMs-d_1 remained and such that any dark reaction would be negligible. ^1H NMR analysis suggested that the product mixture contained 41% **1**-$NHCOCH_3$-d_1, 35% **2**-OMs-d_1, 7% **1**-OMs-d_1, and 17% **3**-OMs-d_1, results consistent with those reported earlier on **2**-OMs itself. Each of the species was separated from its congeners by high-pressure liquid chromatography and each was subjected to ^2H NMR analysis, using a replicated "cut and weigh" procedure.

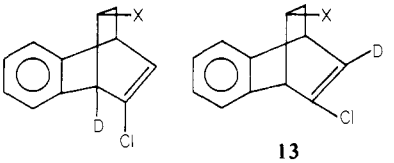
The recovered **2**-OMs-d_1 was, within our limits of measurement, entirely **8**-OMs, that is, there was no suprafacial 1,3-allylic rearrangement, carbocationic or otherwise, which would locate deuterium at C-4 and methanesulfonoxy at C-2. This result suggests that ion **5** (or an analogous radical) is not a primary product of the photochemical process. The amide **1**-$NHCOCH_3$-d_1 comprised a mixture of 60% **10**-$NHCOCH_3$ and 40% **11**-$NHCOCH_3$, while both of the rearranged methanesulfonates had significantly less deuterium mixing. Thus the **1**-OMs-d_1 was 87% **10**-OMs and 13% **11**-OMs and the **3**-OMs-d_1 was 86% **12**-OMs and 14% **13**-OMs.

In order to rule out the possibility that these distributions were the result of some unexpectedly large isotope effect rather than those of mechanism, we studied a mixture of **2**-OMs and **9**-OMs.

(8) Cristol, S. J.; Strom, R. M. *J. Am. Chem. Soc.* **1979**, *101*, 5707.
(9) Triplet sensitization of a variety of **1**-X and **2**-X compounds, including both poor and good nucleofugal groups, led to di-π-methane rearrangements and not to carbon–X bond cleavage.
(10) This reagent/solvent system has been used[11] in the conversion of deuterated *endo*-2-norbornyl *p*-bromobenzenesulfonate to *exo*-norbornyl acetate without deuterium scrambling, that is, without carbenium-ion intervention.
(11) (a) Murr, B. L.; Conkling, J. A. *J. Am. Chem. Soc.* **1970**, *92*, 3462. (b) Maskill, H. *Ibid.* **1976**, *98*, 8482.
(12) We estimate that our deuterium analytical results are good to ±5%.

This was prepared[8] by acetolysis of **8**-OMs, which gave a 60:40 mixture of **8**-OAc and **9**-OAc. Conversion of this mixture to alcohol, followed by oxidation to ketone, reduction with lithium aluminum hydride, and esterification, gave a mixture containing ca. 60% **2**-OMs and 40% **9**-OMs. Although this mixture was largely **2**-OMs, the ^2H NMR probe is, of course, blind to nuclei other than deuterium, so that it was as useful as pure **9**-OMs. Irradiation, followed by isolation and weighing, gave 33% **2**-OMs-$d_{0.4}$, 8% **1**-OMs-$d_{0.4}$, 23% **3**-OMs-$d_{0.4}$, and 36% **1**-$NHCOCH_3$-$d_{0.4}$.

Now the **2**-OMs-*d* was entirely **9**-OMs (no **8**-OMs observed), again showing no return with rearrangement on the endo side of the molecule. The **1**-$NHCOCH_3$-*d* was 43% **10** and 57% **11** isomer, the **1**-OMs-*d* was 17% **10** and 83% **11**, and the **3**-OMs-*d* was also 17% **12** and 83% **13**. Obviously, then, the results with



12 **13**

8-OMs and **9**-OMs are compatible with the idea of mechanistic differences rather than isotope effect controlled ones.

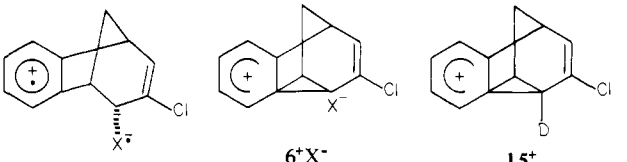
To summarize, the photosolvolysis product was the result of 60% attack at the initial site of the nucleofuge and 40% at the allylic isomer site. The photorearrangement products were the result of about a 5:1 preference for return from the exo face at the original site of the nucleofuge to give **1**-OMS, or return with ring migration to the original site to give **3**-OMs over the analogous returns to the opposite end (allylic end) of the molecule.

When the 83:17 mixture of **10**-OMs and **11**-OMs was irradiated in acetonitrile until 31% of **10**-OMs-d_1 remained, only a trace of **2**-OMs-d_1 was formed. The product mixture contained about equivalent amounts of **1**-$NHCOCH_3$-d_1, **1**-OMs-d_1, and **3**-OMs-d_1. Again the deuterium analysis of the amide showed significant scambling (57% **10**-$NHCOCH_3$ and 43% **11**-$NHCOCH_3$), while the methanesulfonates were significantly less scrambled, **1**-OMs-d_1 being 74% **10** and 26% **11** and **3**-OMs-d_1 being 74% **12** and 26% **13**. Just as does the endo isomer, the *exo*-methanesulfonate gives about 85% reaction at the original allylic site of the nucleofuge and 15% reaction with allylic inversion in the isomerization reactions. There is little exo–endo interconversion, but, unlike the endo isomer, the exo isomer gives some return to its allylic isomer.

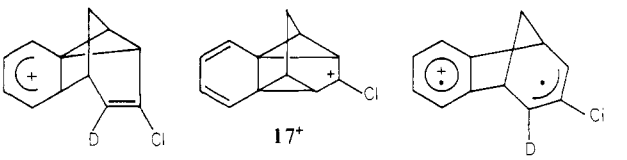
While these, and our previous experiments, do not permit a complete understanding of our system, it is clear that the photorearrangements of **2**-OMs to **1**-OMs and to **3**-OMs involve a much less symmetrical intermediate than do either ground-state solvolyses or the photochemical solvolysis to **1**-$NHCOCH_3$. The photosolvolysis results are consistent with the reaction of an unsymmetrical ion pair involving the allylic cation **5** and the methanesulfonate ion still located more closely to its original carbon atom, which equilibrates with its allylic isomeric ion pair at a rate comparable with its capture by solvent,[8,13] a reaction paralleling the ground-state behavior. No such possibility exists for the formation of **3**-OMs, as we know from ground-state results that 5^+OMs^- gives no **3**-OMs (photochemical processes of reasonable efficiency are like kinetically controlled experiments, as they do not allow for repeated formation of intermediates). A plausible reaction path might involve a twisted allyl cation, in which overlap of the π orbital of the double bond and the p orbital of the cationic center is imperfect. The isomerization of such a twisted ion to 6^+ might be exergonic and might even occur from either end of the allylic system, although not with identical efficiencies. Irradiation with 254-nm light provides about 110 kcal/mol of energy into the molecule, well above that required for bond heterolysis in a solvent of reasonable ionizing power, if one assumes that ground-state ions are produced. Thus heterolytic bond dissociation might readily occur without the formation of a fully delocalized allylic cation. Such a process would not require any participation of the migrating benzene ring in the heterolysis, that is, such a process could yield 6^+ either with net inversion (i.e., from **1**-X) or retention (i.e., from **2**-X). We would propose that the ion pair 6^+X^- then collapses to **1**-X or to **3**-X, or becomes a solvent-separated ion pair. During the time required for the transformation from the tight and relatively unsolvated ion pair to the solvated ion pair, relaxation[14] from 6^+ to 5^+ should occur such that solvolysis products arise from 5^+.

In our previous communication, we noted that irradiation might produce an ion pair 6^+X^- directly from the excited state, without the intervention of an allylic cation. We proposed that the light absorption occurred in the benzene-ring chromophore to give the π–π^* state of the reactant. Intramolecular electron transfer might then occur to give a state best represented as a zwitterionic biradical **14**. That species should be unstable with respect to 6^+X^-, and might be expected to have minimal stereochemical requirements for the transformation, as it involves the attack of an incipient radical center on the unfilled π orbital of the benzene ring. Such a process for producing 6^+ does not allow for any deuterium mixing, so that an alternative path must still be considered for that mixing. The transformation of 15^+ to its isomer 16^+ would accommodate that requirement. While the concerted transformation from 15^+ to 16^+ is probably not symmetry allowed, a path via 17^+ would be.

An alternative for deuterium scrambling would be the formation of the cation biradical **18** which could decay to 15^+ and 16^+ by electron pairing.



14 6^+X^- 15^+



16 17^+ **18**

Experimental Section

General. Preparation of starting materials and analytical instrumentation and methods have been described previously.[8]

Preparation of 2-Deuterio-3-chloro-6,7-benzobicyclo[3.2.1]octa-3,6-dien-exo-2-ol Methanesulfonate (10-OMs). A solution of 1.6 g (5.3 mmol) of tetra-*n*-butylammonium acetate and 528 mg (1.85 mmol) of **8**-OMs in 185 mL of benzene was prepared. The benzene was then removed (in part) by distillation until ca. 40 mL remained. The solution was then heated at reflux for 4 days. The resulting solution was washed with water and brine and dried ($MgSO_4$). Removal of solvent by distillation left an oil (560 mg) whose ^1H NMR spectrum indicated ca. 60% conversion to the deuterium-labeled acetate **1**-OAc-d_1. This acetate was separated from starting material by thick layer chromatography (silica gel–10% ethyl ether in hexane). Ester interchange of the acetate was carried out with 0.1 M sodium methoxide in methanol as described previously.[8] The resulting alcohol was esterified with methanesulfonyl chloride by the procedure described earlier.[8] ^1H and ^2H NMR spectra of the resulting sulfonate ester were consistent with those anticipated for a mixture comprised of 83% **10**-OMs and 17% **11**-OMs.

Direct Irradiation of 8-OMs. A solution of **8**-OMs (259 mg, 0.907 mmol) in 7 mL of dry acetonitrile was placed in a 10-mm (i.d.) quartz tube. The solution was purged with nitrogen and the tube sealed. Irradiation (254 nm) in the "small Rayonet"[8] for 2.75 h at ca. 30 °C resulted in a pale yellow solution. This solution was diluted with water,

(13) Sneen, R. A.; Bradley, W. A. *J. Am. Chem. Soc.* **1972**, *94*, 6975. Sneen, R. A.; Kay, P. S. *Ibid.* **1972**, *94*, 6983. Sneen, R. A.; Carter, J. V. *Ibid.* **1972**, *94*, 6990.

(14) For analogous ground-state situations, see: Goering, H. L.; Humski, K. *J. Am. Chem. Soc.* **1968**, *90*, 6213. Goering, H. L.; Clevenger, J. V. *Ibid.* **1972**, *94*, 1010.

extracted with either, dried ($MgSO_4$), and evaporated to dryness, resulting in 256 mg of yellow oil whose ^1H NMR spectrum was consistent with the analysis reported in the Discussion section. Separation of individual components by LC[8] followed by ^2H NMR analysis gave the results also reported in the earlier part of this paper. When the photoreaction was carried out at 73 °C, the results were identical, within experimental error.

Direct Irradiation of 9-OMs. A solution[8] of 185 mg (0.683 mmol) of a mixture of 40% **9**-OMs and 60% **2**-OMs in 5.25 mL of dry acetonitrile was deoxygenated and irradiated (254 nm) as above for 2.75 h. The reaction mixture was worked up and analyzed as above, giving the product ratios described in the earlier section.

Direct Irradiation of 10-OMs. A 83:17 mixture of **10**-OMs-**11**-OMs (110 mg, 0.38 mmol) in 1.1 mL of dry acetonitrile was placed in a 5-mm quartz NMR tube and deoxygenated as above. The tube was sealed and irradiated (254 nm) in the "Photoprep" apparatus[8] for 15.25 h. Workup as above followed by LC[8] analysis and separation gave the product ratio described in the earlier section, and ^2H NMR analysis gave the deuterium ratios described above.

Acknowledgment. This work was supported by Grant CHE-77-20854 from the National Science Foundation.

Thermorubin. 1. Structure Studies

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Contribution from the Departments of Pharmacological Sciences and Chemistry, State University of New York at Stony Brook, Stony Brook, New York 11794. *Received June 7, 1979*

Abstract: The structure of thermorubin, thought previously to be 5,7-dihydroxy-3,6-dimethoxy-2-[1′,3′-dihydroxy-2′-methoxycarbonyl-9′-xanthon-4′-yl)methyl]anthracene, is shown by physical and chemical evidence to be incorrect. A new structure, namely, 12-hydroxy-10,11-dimethoxy-3-methoxycarbonyl-8-carboxymethyl-9-[1′-oxo-3′-hydroxy-3′-(2″-hydroxyphenyl)-prop-2′-enyl]-1*H*-2-oxanaphthacen-1-one, has been determined largely by an X-ray diffraction analysis, and this accounts for all of the previously observed chemical degradation results. The structure of trimethylthermorubin, obtained by simple methylation of thermorubin, is also elucidated.

I. Introduction

In 1964 Craveri, Coronelli, Pagani, and Sensi[1] described a new thermophilic actinomycete, *Thermoactinomyces antibioticus*, which in submerged culture produced a novel antibiotic substance. This material, termed thermorubin, proved to be very active against Gram-positive bacteria, less active against Gram-negative bacteria, and virtually inactive against yeasts and filamentous fungi. A group[2] from the same organization subsequently investigated the mechanism of antibiotic action and found that thermorubin is bacteriostatic and inhibits protein synthesis at the level of translation, but that DNA and RNA syntheses are unaffected. They reported that, in vitro, thermorubin inhibits protein synthesis directed by natural messenger RNA, but not the synthesis of poly-Phe directed by the synthetic messenger poly-U. The binding of the initiator of protein synthesis, fmet-$tRNA^{met}$, to 70S ribosomes is inhibited, but the reaction of puromycin (an analogue of aminoacyl-tRNA) with initiator already bound to the ribosome is not impaired. In a related study Wishnia and Lin[3] have found, however, that thermorubin does not inhibit the association of this initiator with the 30S ribosomal subunit. Thus thermorubin becomes the first example of an inhibitor which prevents initiator attachment *only* when the 30S and 50S subunits are in association. The latter investigations have also demonstrated that thermorubin very strongly inhibits dissociation of 70S ribosomes. One possible mechanism of bacteriostatic action might be the prevention of the return of the 30S subunits to the common pool. Thermorubin shows no cross-resistance with other known antibiotics and this, together with its seemingly low toxicity (LD_{50} = 300 mg/kg intraperitoneally in mice), would appear to make it a likely candidate for development as an agent for therapeutic use in man. A drawback is that it has very low solubility in physiological fluids, and its activity in vitro is diminished in the presence of animal serum. Nevertheless, it affords 100% protection to mice against *Staphylococcus aureus* infections when administered intraperitoneally for 3 days at the level of 3 mg/kg body weight. The reason for the selective action of thermorubin against procaryotic cells is unknown at this time.

These pharmacological properties form the basis for our continued interest in the structure and in the chemical modification of this antibiotic.

Table I. Comparison of NMR Data for the Four Coupled Protons

compd in C_6D_6	hydrogen resonance position,[a] δ			
	$H_{5'}$	$H_{6'}$	$H_{7'}$	$H_{8'}$
2[b]	6.96	7.18	6.68	7.79
3	6.80	7.04	7.04	8.12

[a] Each of these absorptions is a complex but fairly symmetrical multiplet; assignment of the positions was made on the basis of decoupling experiments already described.[5] [b] A pictorial view of this region of the NMR spectrum has already been published.[5]

II. Previous Structure Studies

Initially, when fermentation broth of *Thermoactinomyces antibioticus* was extracted with organic solvents, the crude material was thought to comprise largely a single product (70–80%). This, after recrystallization from chloroform, afforded the chloroform solvate of thermorubin as a bright red powder. The early studies[1] claimed that thermorubin (a) is optically active, (b) has a molecular weight of 432, (c) has the empirical formula $C_{22}H_{18}O_8$, (d) contains two weakly acidic groups, and (e) has two methoxy groups and an *o*-hydroxyquinone moiety (infrared data). Proof of the presence of the latter group was adduced by the observation that thermorubin was decolorized by a variety of reducing agents. In addition, it was claimed that thermorubin gives a diacetyl derivative when treated with acetic anhydride/pyridine, that it reacts[4] with diazomethane to give a trimethyl ether, and that, on fusion with sodium hydroxide, it yields[4] salicylic acid.

(1) Craveri, R.; Coronelli, C.; Pagani, H.; Sensi, P. *Clin. Med.* **1964**, *71*, 511-522.
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0002-7863/80/1502-5580$01.00/0