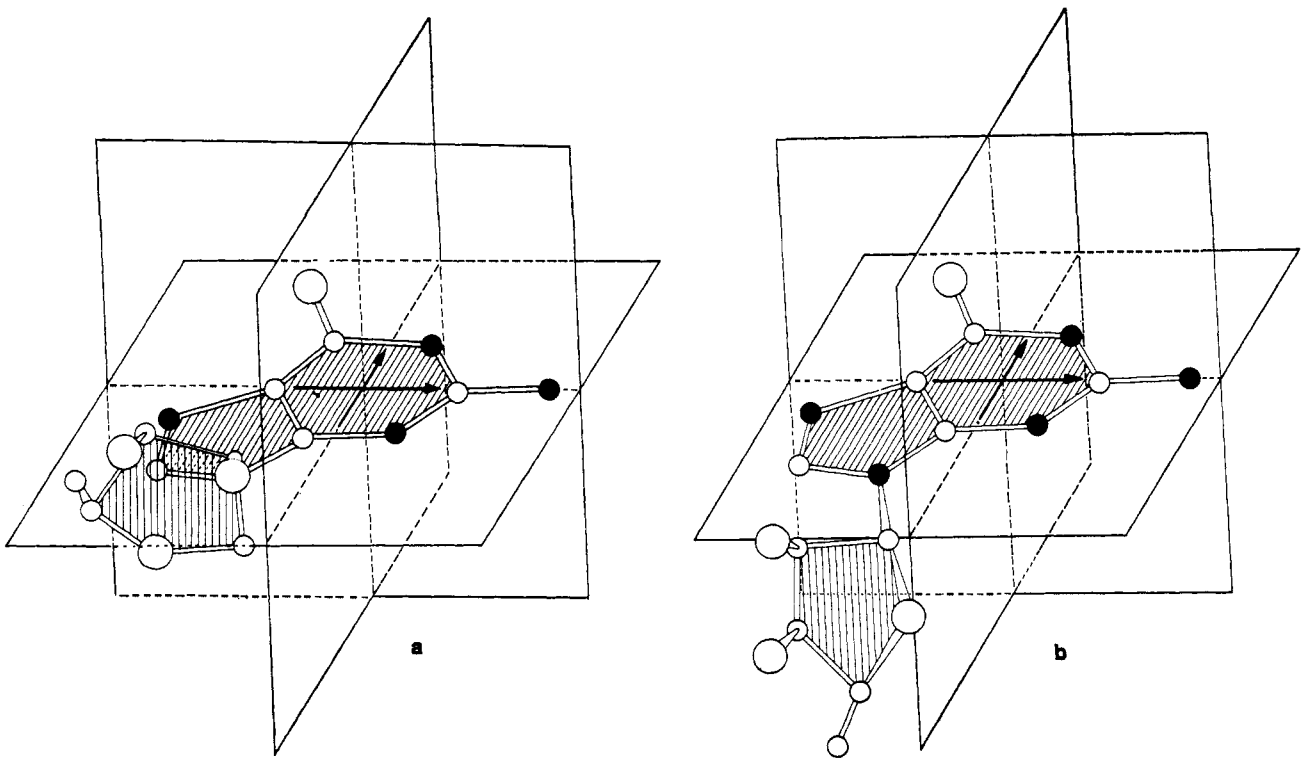

Figure 12. Guanosine aligned along the B_{2u} transition: $\Phi_{CN} = -0°$ (A); $\Phi_{CN} = 90°$ (B).

activity need not be detectable, if the sugar residue is located near a nodal plane.

It also has been suggested[9] that a two state model with the syn and anti conformations as extrema, eliminating more than 75% of all possible conformational ranges, could explain the solvent effects on CD spectra. The conclusions were obtained by comparison with cyclonucleosides.[8,9] But there are several lines of evidence[14-17] showing that the unhindered nucleosides, which can rotate almost freely, can assume all angles Φ_{CN}. The relative amount of these populations can change with various conditions, like the pH or the substituents; solvent effects have to be added to this list of variables.

This implies that a CD band is the algebraic sum of an infinity of signals each of which corresponds to an angle Φ_{CN}; it is therefore only rarely possible to associate the sign of a Cotton effect to a given conformation, because it results from a population of different conformers; while part of the syn conformation could have a negative Cotton effect the other one could have a positive one; the sum could be negative or positive.

Transmethylation from Toxoflavines to Nucleophiles

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Abstract: The antibiotic toxoflavine and analogs undergo demethylation with nucleophiles to give the corresponding 1-demethyltoxoflavines (8-demethylfervenulins), while nucleophiles themselves are methylated by the methyl group eliminated. During the reactions, toxoflavine radical anions, novel radical species, were observed. A possible reaction mechanism is proposed for this transmethylation.

The structure of an antibiotic toxoflavine (identical with xanthothricin) having strong physiological activities has been established as 1,6-dimethylpyrimido-[5,4-*e*]-*as*-triazine-5,7(1*H*,6*H*)-dione by total synthesis in 1961.[1] The structural relationship of toxoflavine to riboflavine is particularly remarkable, since both compounds possess similar oxidation–reduction systems. It is also known that the peroxide-generating capacity of toxoflavine may be responsible for its poisonous character.[2] We have now found another

(1) G. D. Daves, R. K. Robins, and C. C. Cheng, *J. Amer. Chem. Soc.*, **83**, 3904 (1961).

(2) H. E. Latuasan and W. Berends, *Biochim. Biophys. Acta*, **52**, 502 (1961).

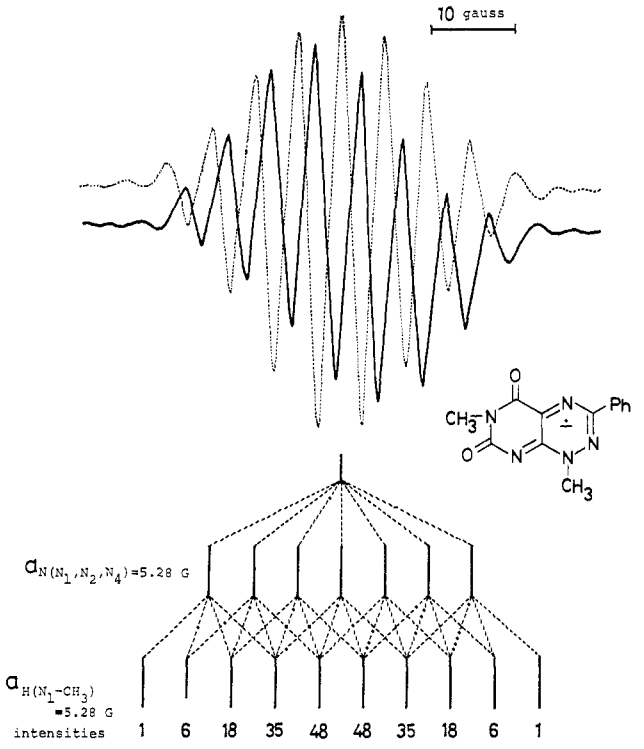


Figure 1. First derivative (boldface) and second derivative (broken line) esr spectra of 3-phenyltoxoflavine radical anion in dimethylformamide and the coupling scheme based on the coupling constants indicated.

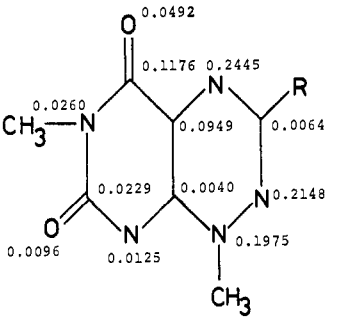


Figure 2. Spin densities of toxoflavine radical anion calculated by Hückel MO method. The parameters of the coulomb and resonance integrals for substituent groups are as follows. For =N–, a_X = 0.6, a_r = 0.1, I = 1; for –N<, a_X = 1, a_r = 0.1, I = 1; for =O, a_X = 2, a_r = 0.2, I = 1.4. a_X is the coulomb integral of the substituent X: $\alpha_X = \alpha + a_X\beta$. a_r is the coulomb integral of the carbon atom adjacent to X: $\alpha_{adj} = \alpha + a_r\beta$. I is the resonance integral between that carbon and X: $\beta_{C-X} = I\beta$.

Table I. Observed Coupling Constants for the Toxoflavine Radical Anions in Dimethylformamide

3-substituent	Coupling constants, G a_N[a]
H	5.24
Ph	5.28
3-Pyridyl	5.27
2-Pyridyl	5.27
2-Thienyl	5.30

[a] $a_N(a_{N_1} \approx a_{N_2} \approx a_{N_4}) \approx a_{H(N_1-CH_3)}$.

striking property of this compound, namely that toxoflavine and its analogs readily undergo demethylation with several nucleophiles to give the corresponding 1-demethyltoxoflavines (8-demethylfervenulins), while nucleophiles themselves were methylated by the methyl group eliminated. This transmethylation would be interesting from a biological point of view and is possibly a useful reaction whose generality heretofore has not been realized. Furthermore, we have observed novel radical species during the reactions. The purpose of the present paper is to propose a possible reaction mechanism of the novel transmethylation reaction; additionally, we wish to describe the structure of the free radical observed by analyzing the esr spectra and by evaluating the pertinent coupling constants.

Heating toxoflavine and analogs (**1**)[3] in dimethylformamide or dimethylacetamide at 80–100° for 5 min under nitrogen (under aerobic conditions also) led to the dark green solutions in which strong esr signals were observed. After 30 min at this temperature or after several hours at room temperature, the color changed to pale yellow, wherewith the esr signals slowly disappeared. Finally 1-demethyltoxoflavine (8-demethylfervenulin)[4] and its analogs (**2**) were separated in almost quantitative yields. From the mother liquor only methanol was detected in yields of 17–25% by gas chromatography.

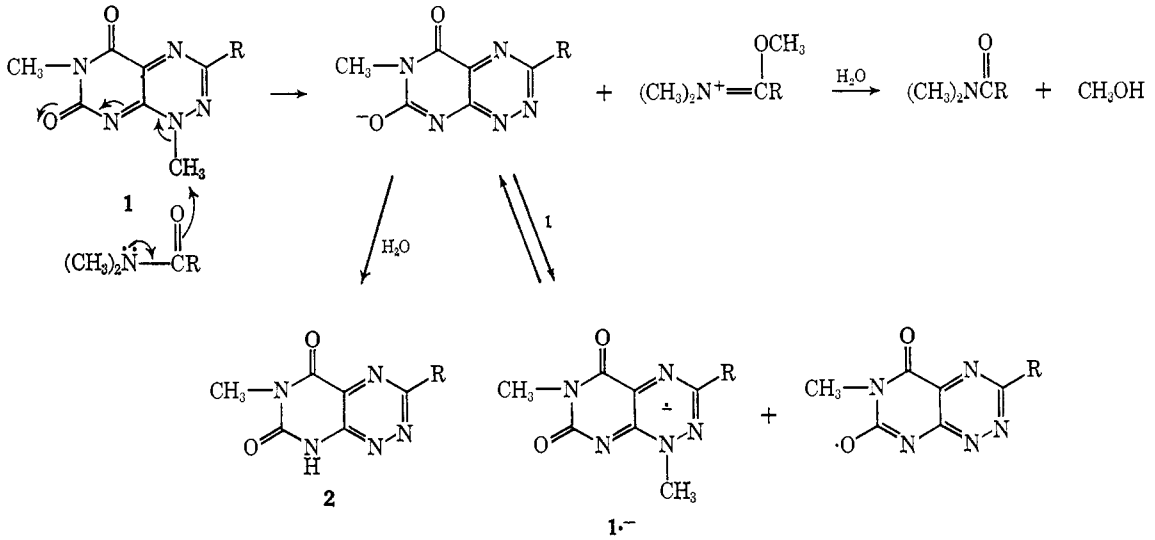
The radicals showed hyperfine structures consisting of ten lines with approximate intensity ratios 1:6:18:35:48:48:35:18:6:1 (Figure 1, Table I). The same esr spectra were obtained by reaction of **1** with potassium *tert*-butoxide in dimethylformamide. Therefore, the observed signals during the transmethylations have proved to be ascribed to toxoflavine radical anions.

The esr spectra can be explained on the basis of the coupling scheme (Figure 1) in which the couplings to the nearly identical three nitrogen atoms are the same as that to the protons of N_1-methyl. It is known that in some examples[5–7] the coupling constant a_H of the NCH_2 group is almost the same as that of a_N; this also appears to be the case in our toxoflavine radical anions. The Hückel LCAO-MO calculation on the toxoflavine radical anion gives the spin densities indicated in Figure 2 and so helps clarify this interpretation. Much smaller coupling constants for the nitrogens of the 6 and 8 position would be expected because of the much smaller spin densities at these positions. Therefore their hyperfine couplings could not be observed under these conditions.

From the above facts, we suggest the mechanism outlined in Scheme I in which dimethylformamide or dimethylacetamide could act as a nucleophile, displacing the conjugate bases of **2**, which are well-stabilized anions. These anions may be good enough reducing agents to be in equilibrium with some toxoflavine radical anions (**1**·⁻). They would protonate from adventitious water to give the **2** observed. On the other hand, dimethylformamide or dimethylacetamide could be methylated into the reactive dimethylformimino ether or dimethylacetimino ether, which

(3) F. Yoneda, K. Shinomura, and S. Nishigaki, *Tetrahedron Lett.*, 851 (1971).
(4) 1-Demethyltoxoflavine has been prepared in multiple steps: T. K. Liao, F. Baiocchi, and C. C. Cheng, *J. Org. Chem.*, 31, 900 (1966).
(5) J. Q. Adams, S. N. Nicksic, and J. R. Thomas, *J. Chem. Phys.*, 45, 654 (1966).
(6) C. J. W. Gutch and W. A. Waters, *J. Chem. Soc.*, 751 (1965).
(7) G. Chapelet-Letourneux, H. Lemaire, and A. Rassat, *Bull. Soc. Chim. Fr.*, 3283 (1965).

Scheme I



is readily hydrolyzed into methanol and the original solvents.

Next, other nucleophiles have been used for the transmethylation and similar results were obtained. For example, heating **1** in acetic acid at 80–100° for 3 hr gave **2** in quantitative yields, while acetic acid was methylated into methyl acetate, which was detected in yields of about 15% by gas chromatography. In this case a trace of methanol was also detected. When indole derivatives were used as nucleophiles, interesting results were obtained. For example, refluxing 3-phenyltoxoflavine, a member of toxoflavine derivatives, with indole in dioxane for 5–10 hr likewise caused separation of 3-phenyl-1-demethyltoxoflavine in 40% yield. Concentrating the mother liquor *in vacuo*, dissolving the resulting residue in methanol, and allowing the solution to stand overnight in the refrigerator gave a stable 1:1 complex of 3-phenyltoxoflavine and 1-methylindole as dark orange crystals in 30% yield. Fusion of 3-phenyltoxoflavine with indole at 150° for 15 min also gave 3-phenyl-1-demethyltoxoflavine and the complex in 48 and 25% yields, respectively. This complex was unequivocally prepared by mixing equimolar ratios of 3-phenyltoxoflavine and 1-methylindole in dioxane. This fact clearly shows that the methyl group of a toxoflavine intermolecularly transferrred to the 1 position of indole to give 1-methylindole, which was captured by unchanged toxoflavine to form the complex.[8]

Experimental Section

Materials. Toxoflavine and its analogs were synthesized by the nitrosative cyclization of the hydrazones of 3-methyl-6-(1′-methylhydrazino)uracil.[3] 1-Methylindole was prepared according to the literature.[9] The potassium *tert*-butoxide was commercial material (E. Merck Co.). The other reagents used were all of a GR grade.

Measurement of the Esr Spectra. Esr measurements were carried out at room temperature using a JEOL P-10 apparatus at the X-band and 100 kcps field modulation.

All the radical anions were prepared in dimethylformamide or dimethylacetamide by heating at 80–100° for 5–10 min. After being cooled, the resulting dark green solutions gave strong esr absorptions. The same radical anions were formed by adding *tert*-butyl alcohol solution containing an excess of potassium *tert*-butoxide to a dimethylformamide solution of toxoflavines.

Transmethylation from 3-Phenyltoxoflavine to Indole. A solution of 1 g (0.0037 mol) of 3-phenyltoxoflavine and 0.43 g (0.0037 mol) of indole in 20 ml of dioxane was heated under reflux for 5 to 10 hr. After being cooled, the crystals which separated were collected by filtration, washed with methanol, and dried to give 0.38 g (40%) of 3-phenyl-1-demethyltoxoflavine. The filtrate was concentrated *in vacuo* and the resulting syrup was allowed to stand overnight in the refrigerator to give dark red crystals, which were collected by filtration, washed with water, and recrystallized from methanol to give 0.44 g (30%) of dark orange to brown crystals of the complex of 3-phenyltoxoflavine and 1-methylindole, mp 159–166°.

Anal. Calcd for $C_{22}H_{20}N_6O_2$: C, 65.98; H, 5.03; N, 20.99. Found: C, 66.14; H, 5.20; N, 20.59.

Complex Formation from 3-Phenyltoxoflavine and 1-Methylindole. To a solution of 0.2 g (0.0015 mol) of 1-methylindole in 10 ml of ethanol was added 0.27 g (0.001 mol) of 3-phenyltoxoflavine. The dark red solution was allowed to stand overnight at room temperature and the precipitates were filtered off. The filtrate was evaporated under reduced pressure and the residue was washed with water to give dark brown powder. Recrystallization from methanol gave brown crystals which were, in all respects, with the complex described above.

Acknowledgment. The authors are grateful to Dr. K. Ōhigashi for measurement of esr spectra.

(8) Indole itself does not form such a complex with a toxoflavine.

(9) K. T. Potts and J. E. Saxton, *Org. Syn.*, **40**, 68 (1960).