Fig. 1.—Apparent s-character of Sn atomic orbital in Sn–C bond in tetramethyltin and the methyltin halides.

bond. This relationship was first pointed out by Karplus and Grant[7] and appears to hold remarkably well in the case of ^{13}C—H coupling in the series methane–ethylene–acetylene reported recently by Shoolery,[8] in spite of certain assumptions implicit in this treatment. The coupling constant A_{13C-H} varies linearly with the per cent. s-character in the bonding orbitals on carbon, and *extrapolates to zero* coupling for a pure p_σ orbital on carbon. Muller and Pritchard[9] also have related A_{13C-H} values observed in hydrocarbons and substituted methanes to differences in the hybridization of the atomic orbitals on carbon.

We believe a similar relationship, that shown in Fig. 1, exists between the tin–proton couplings reported here and the hybridization of the atomic orbitals on tin. The line in Fig. 1 is drawn with the assumption that the contact mechanism makes the dominant contribution to Sn-C-H coupling and that, except for rehybridization of the tin atom, other components of the bonding system Sn–C–H do not change appreciably. This latter assumption receives some support from our separate observation that the ^{13}C–H spin–spin interactions within the methyl groups remain nearly constant in the series of compounds considered here.

Taking as one point the observed coupling constant for tetramethyltin as representing 25% s-character and the origin as the second point, we have generated the line in Fig. 1. When the other coupling constants (Table I) are placed on this line, the distribution of points indicates considerable rehybridization in the tin atom in methyltin halide molecules. For the case of aqueous solutions of methyltin halides, presumed to contain the cor-

responding methyltin cations, almost complete rehybridization is indicated. This result in particular is an agreement with the conclusions of Okawara, Webster and Rochow,[1b] based on the interpretation of their infrared data, with respect to the configuration of methyltin cations.

We wish to emphasize the fact that the linear relationship assumed in Fig. 1 does not have, at present, as firm a theoretical basis as in the case of directly bonded atoms[7] and must be presented here as an interesting correlation. Measurements of the ^{13}C–Sn couplings in these compounds would give a more direct indication of the rehybridization of the tin atom and provide further justification of our assumptions about other changes in the Sn–C–H bonding system.

Preliminary results on the vinyltin halides indicate similar behavior in that series of compounds. Work on the vinyl series and related Group IVb compounds is in progress and will be published in the near future.

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THE TOTAL SYNTHESIS OF TOXOFLAVIN

Sir:

Toxoflavin, a yellow crystalline solid isolated in 1933 from *Pseudomonas cocovenenans*, has been shown to be responsible for certain fatal food poisoning among the natives of Java.[1,2] This compound has stimulated considerable interest[3] because of its high toxicity and potent antibiotic activity.[3h] The structure of toxoflavin was originally proposed by van Veen and Baars[3c] as I (desmotropic tautomer[4] of 1-methylxanthine), but Johnson and Ambelang,[3e] and Nugteren[3f] demonstrated the implausibility of this structure. Recently, van Damme, *et al.*,[3h] reinvestigated the structure of toxoflavin and proposed it as either 1,6-dimethyl-5,7-dioxo-1,5,6,7-tetrahydropyrimido[5,4-e]-*as*-triazine (II) or its structural isomer, IIa. These investigators preferred structure II on the basis of degradation and X-ray diffraction studies.[3i,3j]

(1) A. G. van Veen and W. K. Mertens, *Proc. Akad. Wetenschappen Amsterdam*, **36**, 666 (1933); *Rec. trav. chim.*, **53**, 257, 398 (1934).

(2) W. K. Mertens and A. G. van Veen, *Geneesk. Tijdsdhr. Ned. Indië*, **73**, 1223, 1309 (1933); *Meded. Dienst Volksgezondheid Ned. Indië*, **22**, 209 (1933).

(3) (a) D. Amar and A. Grevenstuk, *Geneesk. Tijdschr. Ned. Indië*, **75**, 104 (1935); (b) K. G. Stern, *Biochem. J.*, **29**, 500 (1935); (c) A. G. van Veen and J. K. Baars, *Proc. Akad. Wetenschappen Amsterdam*, **40**, 498 (1937); (d) A. G. van Veen and J. K. Baars, *Rec. trav. chim.*, **57**, 248 (1938); (e) T. B. Johnson and J. C. Ambelang, *J. Am. Chem. Soc.*, **61**, 2483 (1939); (f) D. H. Nugteren, Thesis, Delft, 1956; (g) D. H. Nugteren and W. Berends, *Rec. trav. chim.*, **76**, 13 (1957); (h) P. A. van Damme, A. G. Johannes, H. C. Cox and W. Berends, *Rec. trav. chim.*, **79**, 255 (1960); (i) H. E. Latuasan and W. Berends, "The Origin of the Toxicity of Toxoflavin," *Biochem. Biophys. Acta*, in press; (j) A. S. Hellendoorn, R. M. T. Cate-Dhont and A. F. Peerdeman, *Rec. trav. chim.*, **80**, 307 (1961).

(4) H. Blitz, *J. prakt. Chem.*, **146**, 83 (1936).

(7) M. Karplus and D. M. Grant, *Proc. Nat. Acad. Sci.*, **45**, 1269 (1959).

(8) J. N. Shoolery, *J. Chem. Phys.*, **30**, 1427 (1959).

(9) N. Muller and D. E. Pritchard, *ibid.*, pp. 768, 1471.
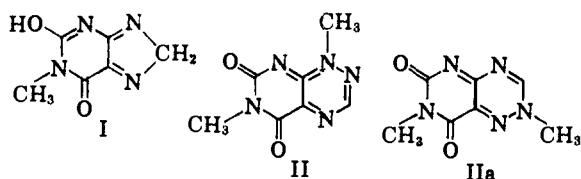
We now wish to report the total synthesis of toxoflavin which confirms structure II in every respect. 2-Thiobarbituric acid[5] (III) was converted to 6-hydroxy-3-methyl-2-(methylthio)-4-(3H)-pyrimidinone (IV), m.p. 195–197° (calcd.: C, 41.8; H, 4.6; N, 16.3. Found: C, 42.1; H, 4.6; N, 16.0) via methylation. Chlorination of IV with phosphorus oxychloride in the presence of N,N-dimethylaniline
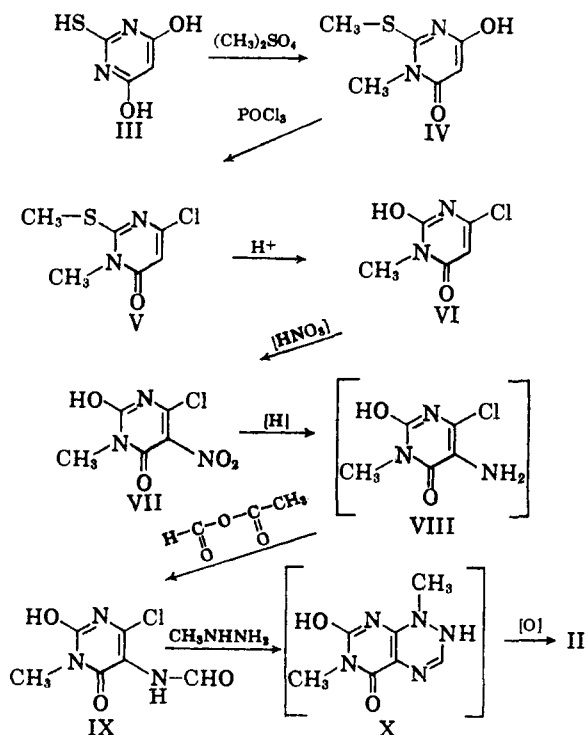
gave the corresponding chloro derivative (V) m.p. 111–112°, in 57% yield[6] (calcd.: C, 37.9; H, 3.7; N, 14.7. Found: C, 37.6; H, 3.8; N, 14.6). Compound V was hydrolyzed in dilute acid to yield 31–47% of 6-chloro-2-hydroxy-3-methyl-4(3H)-pyrimidinone (VI), m.p. 277–279° (calcd.: C, 37.5; H, 3.1; N, 17.4. Found: C, 37.3; H, 3.3; N, 17.4), which was nitrated readily below 25° to give 6-chloro-2-hydroxy-3-methyl-5-nitro-4(3H)-pyrimidinone (VII), m.p. 195–197°, in 45% yield (calcd.: C, 29.3; H, 2.0; N, 20.5. Found: C, 28.8; H, 2.1; N, 20.2). Catalytic reduction of VII in methanol containing a small amount of aqueous ammonia, and then addition of formic–acetic anhydride to the concentrated reaction mixture gave 6-chloro-5-formamido-2-hydroxy-3-methyl-4(3H)-pyrimidinone (IX), m.p. 225–226°, in 43% yield (calcd.: C, 35.5; H, 3.0; N, 20.7. Found: C, 35.4; H, 3.2; H, 20.8). Compound IX then was refluxed with one equivalent of methylhydrazine in ethanol. The resulting precipitate was dissolved in a satu-

(5) H. Michael, J. prakt. Chem., [2] 35, 456 (1887).

(6) All yields are calculated after purification.

rated ammonium sulfate solution, which was extracted with chloroform. Evaporation of the chloroform extract and recrystallization of the crude product from propanol-1 gave a 28% yield of toxoflavin, m.p. 172–173° dec., (lit.[3c] m.p. 171° dec.) as bright yellow plates (calcd.: C, 43.5; H, 3.7; N, 36.3. Found: C, 43.7; H, 3.7; N, 36.1).

The synthetic toxoflavin exhibited identical ultraviolet absorption [$\lambda_{max}^{pH\ 1.7}$ 257.5 mμ, (ϵ 16,400); 394 mμ, (ϵ 2,500)] and infrared absorption [λ_{max}^{KBr} (μ) 3.4 (w), 5.9 (s), 6.0 (s), 6.25 (s), 6.6 (s), 6.95 (in), 7.05 (m), 7.25 (w), 7.4(w), 7.8 (s), 8.1 (s), 8.35 (w), 8.8 (m), 9.6 (m), 10.4 (w), 10.9 (s), 11.55 (w), 12.35 (m), 13.0 (s), 13.8 (m) and 14.15 (m)] with that of the natural product.[3b,d,h]

Latuasan and Berends[3i] noted that toxoflavin apparently was identical with the antibiotic xanthothricin isolated by Machlowitz, et al.[7a] from a culture of a member of the genus Streptomyces. Comparison of an authentic sample of xanthothricin[7b] with synthetic toxoflavin spectrographically and in paper chromatographic systems confirmed this identity. (R_f values of both toxoflavin and xanthothricin in 95% ethanol and in 1-butanol–10% urea are 0.35 and 0.29, respectively.)

The present synthetic route should readily provide related compounds possessing interesting biological properties. An extension of this work currently is in progress. This study was made possible under the auspices of the Cancer Chemotherapy National Service Center of the National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-3025.

(7) (a) R. A. Machlowitz, W. P. Fisher, B. S. McKay, A. A. Tytell and J. Charney, "Antibiotics and Chemotherapy," 4, 259 (1954); (b) this comparison was made possible by a generous gift of xanthothricin kindly provided by Dr. Frank J. Wolfe of Merck and Company, Inc., Rahway, New Jersey, to whom sincere thanks are due.

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A REVERSIBLE COMPLEX OF COBALTODIHISTIDINE WITH MOLECULAR NITRIC OXIDE

Sir:

Cobaltodihistidine (CoΦ_2) reversibly links nitric oxide in the molecular ratio 1:1 according to the reaction CoΦ_2 + NO $\rightleftarrows$ CoΦ_2NO, the stability constant of which, as determined by the measurements of the partial pressure of nitric oxide, has a value of approximately 10^4 at 20°. A solution 10^{-3} F of CoΦ_2 is practically colorless; it turns violet by adding nitric oxide and becomes colorless again by adding N$_2$. The violet solution exhibits maximum absorption at 455 mμ with a molar extinction coefficient of about 75. Solutions of CoΦ_2NO, acidified by perchloric acid, release nitric oxide quantitatively, and cobalt is found to be again in the bivalent state.

The addition and removal cycles of nitric oxide may be repeated many times in succession, but the quantity of nitric oxide added or removed diminishes after each cycle and simultaneously some oxidation to CoΦ_2^+ occurs. The oxidized form CoΦ_2^+ does not link nitric oxide. The be-