retention of ^{14}C but complete loss of tritium, thereby establishing the precursor relationship of loganic acid (**1**) to gentiopicroside (**5**).[16]

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Biosynthesis of the Tetracyclines. XII.[1] Anhydrodemethylchlortetracycline from a Mutant of *Streptomyces aureofaciens*

Sir:

We recently reported the isolation of 4-aminodedimethylaminoanhydrodemethylchlortetracycline.[2] We commented at that time that no anhydrotetracycline derivative having the intact dimethylamino group had yet been observed as a product of a blocked mutant of *Streptomyces aureofaciens*. We have continued to search, however, for such a mutant, and now report a successful achievement of this goal. Anhydrodemethylchlortetracycline was successfully isolated from mutant 1E6113, a derivative of a demethylchlortetracycline-producing parental strain.[3] Mutant 1E6113 normally produces a small quantity of demethylchlortetracycline (DMCT) (about 10–25 μg/ml), but in the absorption spectrum of an acidic aqueous extract there was seen a significant amount of absorption in the 420–440 mμ region where the anhydrotetracyclines show a moderately strong maximum. Using the absorbancy in this region as a guide, we were able to isolate the substance responsible and to show that it was identical with an authentic specimen of anhydrodemethylchlortetracycline.[4] The isolation proceeded as follows: 1 l. of 1E6113 fermented mash (microbiological assay 25 μg/ml as DMCT) was acidified to pH 1.5 with perchloric acid, washed with hexane (centrifugation), and extracted with ethyl acetate (centrifugation). The extract, which by paper chromatographic examination contained essentially no demethylchlortetracycline, was concentrated to dryness and the residue, 840 mg, taken up in tetrahydrofuran (THF). The solution was dried onto 30 g of diatomaceous earth and packed onto the top of a 3 in. × 12 in. partition chromatographic column consisting of 300 g of diatomaceous earth filter aid which had been thoroughly mixed with 150 ml of 0.1 M ethylenediaminetetraacetic acid (EDTA) buffer at pH 8.3. The column was developed with chloroform and the eluate collected in 100-ml fractions. Spectrophotometric examination of the fractions showed anhydrotetracycline-like absorption in cuts 8 through 20. These were combined and evaporated to dryness to yield 80 mg of a partly crystalline product, identified as predominantly amphoteric anhydrodemethylchlortetracycline by paper chromatography (orange fluorescing spot at R_f 0.48 in pH 8.3 EDTA–butanol system). The compound, 80 mg, was converted to the hydrochloride by dissolving in 0.5 ml of 1-butanol plus 0.3 ml of 8 N hydrochloric acid. The crystalline hydrochloride, 53 mg, was shown to be pure anhydrodemethylchlortetracycline by exact correspondence of the ultraviolet and infrared spectra with those of an authentic specimen.[4] Paper chromatographic behavior, in two systems, of the isolated compound likewise was identical with that of the authentic specimen.

Although the original 1E6113 mash contained a small amount of demethylchlortetracycline, we took pains in the isolation to avoid conditions which might partly or completely dehydrate this compound to the anhydro derivative. In addition, the isolation method used was one which separated the already present anhydro derivative from the parent compound at an early point. Finally, the quantity of anhydrodemethylchlortetracycline isolated in pure form was greater than the total DMCT content of the starting mash. For these reasons we feel that there is no possibility that the anhydrodemethylchlortetracycline is an isolation artifact.

In that part of the biosynthetic pathway to the tetracyclines[5] which involves tetracyclic intermediates, blocked mutants have now been reported for each step, excepting only two. These two steps are: 4-hydroxylation of the pretetramids (a product of this hydroxylation, 4-hydroxy-6-methylpretetramid, is known[6]), and the 12a-hydroxylation of the 4-hydroxypretetramids to yield the 4-ketodedimethylaminoanhydrotetracyclines. Some experimental observations on the latter biosynthetic step will be presented in a forthcoming comunication.

(1) Previous paper in this series: 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).

(2) J. R. D. McCormick, E. R. Jensen, S. Johnson, and N. O. Sjolander, *ibid.*, **90**, 2201 (1968).

(3) Mutant 1E6113 was isolated by Dr. J. Growich and Mr. N. Deduck of these laboratories.

(4) J. S. Webb, R. W. Broshard, D. B. Cosulich, W. J. Stein, and C. F. Wolf, *J. Am. Chem. Soc.*, **79**, 4563 (1957).

(5) J. R. D. McCormick in "Antibiotics," Vol. 2, D. Gottlieb and P. D. Shaw, Ed., Springer, New York, N. Y., 1967.

(6) J. R. D. McCormick, U. H. Joachim, E. R. Jensen, S. Johnson, and N. O. Sjolander, *J. Am. Chem. Soc.*, **87**, 1793 (1965).

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The Reaction of Complexes of Rhodium(I) Chloride with Norbornadiene

Sir:

Norbornadiene is oligomerized by several metal catalysts[1] to dimers and trimers that are related formally to the molecules of the starting hydrocarbon by a simple cycloaddition process.[1h,2,3] The contrasting hypotheses that the new carbon–carbon bonds form simultaneously[1i,4] or sequentially[2] have both been advanced. This communication reports the reaction of

(1) (a) R. Pettit, *J. Am. Chem. Soc.*, **81**, 1266 (1959); (b) C. W. Bird, R. C. Cookson, and J. Hudec, *Chem. Ind.* (London), 20 (1960); (c) C. W. Bird, D. L. Colinese, R. C. Cookson, J. Hudec, and R. O. Williams, *Tetrahedron Letters*, 373 (1961); (d) G. N. Schrauzer and S. Eichler, *Chem. Ber.*, **95**, 2764 (1962); (e) D. R. Arnold, D. J. Trecker, and E. B. Whipple, *J. Am. Chem. Soc.*, 87, 2596 (1965); (f) P. W. Jolly, F. G. A. Stone, and K. Mackenzie, *J. Chem. Soc.*, 6416 (1965); (g) D. M. Lemal and K. S. Shim, *Tetrahedron Letters*, 368 (1961); (h) J. J. Mrowca and T. J. Katz, *J. Am. Chem. Soc.*, **88**, 4012, 5941 (1966); (i) G. N. Schrauzer, B. N. Bastian, and G. A. Fosselius, *ibid.*, **88**, 4890 (1966).

(2) T. J. Katz and N. Acton, *Tetrahedron Letters*, 2601 (1967).

(3) R. Hoffmann and R. B. Woodward, *J. Am, Chem. Soc.*, **87**, 2046 (1965).

(4) F. D. Mango and J. H. Schachtschneider, *ibid.*, **89**, 2484 (1967).

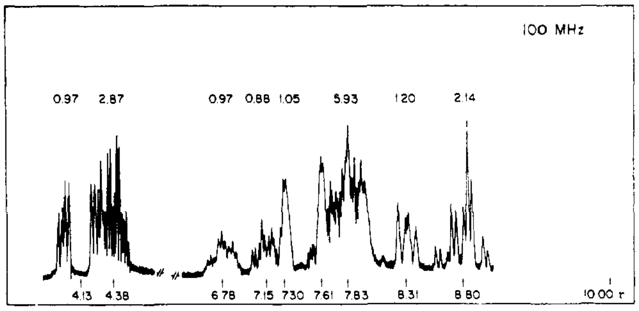
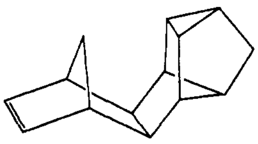


Figure 1. Nmr spectrum of the dimer (in CCl$_4$) assigned structure II. Intensities are above the peaks.

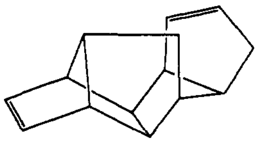
norbornadiene with coordination complexes of rhodium(I) chloride and triphenylphosphine to give dimers and trimers in some of which the carbon skeleton is greatly altered. The new bonds in these products seemingly must arise sequentially.

When norbornadiene (NBD) is refluxed with the three complexes (NBD)[(C$_6$H$_5$)$_3$P]RhCl,[5–7] [(C$_6$H$_5$)$_3$P]$_3$RhCl,[8] or [(C$_6$H$_5$)$_3$P]$_2$(CO)RhCl,[6,9,10] the first complex forms from the other two, and a mixture of dimers (*ca.* 50% yield) also forms, consisting mainly (90%) of similar amounts of two substances, that previously assigned structure I [1h,2,11] and that now assigned structure II.

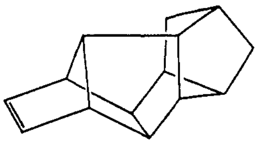
Small amounts of three other isomers have been isolated and characterized: the known caged, saturated dimer,[1c,g] a substance that might be III, and IV, whose structure is not yet determined, but which is pentacyclic and appears to have the functionality -CH(CH$_3$)-.[6]



I



II



III

The dimer to which structure II is assigned is a liquid isolable by distillation.[6] Its nmr (Figure 1), uv, and ir[1h,11] spectra show the presence of two unconjugated double bonds and the absence of a nortricyclene moiety. The hydrocarbon with aqueous silver nitrate gives a complex,[6] C$_{14}$H$_{16}$AgNO$_3$, decomposed by aqueous ammonia to the starting hydrocarbon, whose structure as determined by diffraction of X-rays from a single crystal is shown in Figure 2: C$_{14}$H$_{16}$·AgNO$_3$; mol wt 354.2; orthorhombic, $a = 17.19 \pm 0.03$, $b = 6.41 \pm 0.01$, $c = 11.70 \pm 0.02$ Å; $Z = 4$, space group Pnma

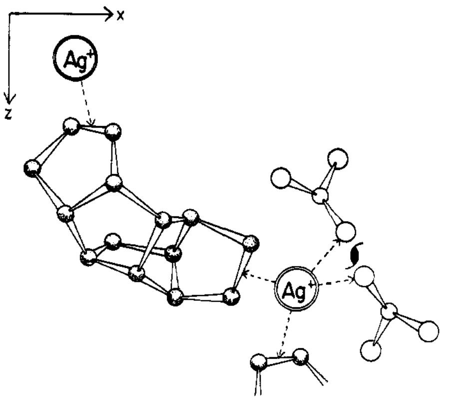
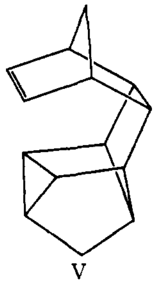


Figure 2. See text.

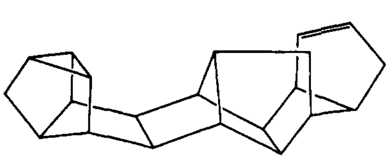
(D$_{2h}$[16]) or Pn2$_1$a (C$_{2v}$[9]). The results of the analysis establish Pn2$_1$a. Two crystals were used to collect 923 independent reflections from equiinclination Weissenberg photographs (Cu Kα) taken at 4°. At the present stage of refinement,[12] with positional and isotropic temperature factors, the *R* value on all observed reflections is 0.16.

The tentative assignment of structure III[6] is based on nmr and ir[1h,11] indications of only one double bond and no nortricyclene and on the observation that when the hydrocarbon assigned structure V[1h,2] is allowed to react with hydriodic acid at 65–70° there is formed, besides the saturated hydrocarbon and hydrogen iodide adduct previously described,[2] another hydrogen iodide adduct (or mixture of isomers)[6] that upon dehydrohalogenation with potassium hydroxide in ethanol[13] gives two isomeric hydrocarbons, one of which is described above and assigned structure III.

A speculation as to the origin of II and III is that they arise from the reaction of a precursor of structure I or V in which the rhodium atom replaces one of the cyclopropyl bonds.[2] However, I or V is not itself an intermediate in the reaction, being inert to [(C$_6$H$_5$)$_3$P]$_3$RhCl, to (NBD)[(C$_6$H$_5$)$_3$P]RhCl, and to a mixture of the former with norbornadiene.



V



VI

Two new hydrocarbon trimers are also formed. They are isolated by chromatography, crystallization, and glpc. The high-field region of the nmr spectrum of the first,[6] mp 118–119.5°, resembles that of I[1h] while the low-field region resembles that of II, suggesting that this trimer is related to II by the addition of a norbornadiene residue as pictured in structure VI. The nmr

(5) M. A. Bennett and G. Wilkinson, *J. Chem. Soc.*, 1418 (1961).

(6) This compound analyzed correctly.

(7) Mp 224.5–225.5°; lit.[5] 163–164°.

(8) (a) J. A. Osborn, F. H. Jardine, J. F. Young, and G. Wilkinson, *J. Chem. Soc., A*, 1711 (1966); (b) M. A. Bennett and P. A. Longstaff, *Chem. Ind.* (London), 846 (1965).

(9) (a) L. Vallarino, *J. Chem. Soc.*, 2287 (1957); (b) J. Chatt and B. L. Shaw, *Chem. Ind.* (London), 290 (1961); (c) A. Rusina and A. A. Vlček, *Nature*, **206**, 295 (1965).

(10) Mp 297–302° dec; lit.[9a] 195–200° dec.

(11) T. J. Katz, J. C. Carnahan, Jr., and R. Boecke, *J. Org. Chem.*, **32**, 1301 (1967).

(12) Full details of the analysis and a description of the structure will be published later.

(13) (a) P. D. Bartlett and I. S. Goldstein, *J. Am. Chem. Soc.*, **69**, 2553 (1947); (b) G. T. Youngblood and P. Wilder, Jr., *J. Org. Chem.*, **21**, 1436 (1956).

of the other trimer (a liquid)[6] suggests its structure is related to that of IV in a similar way.

[14] (a) Supported by National Science Foundation GP 7809; (b) supported by GM 12470 and a Sloan fellowship.

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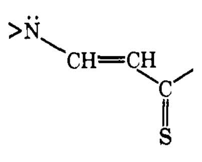
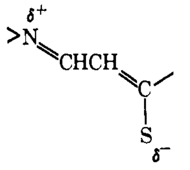
New Synthesis of 2,5-Disubstituted Thiophenes

Sir:

We have described recently an easy preparation of a new class of compounds, dithioacrylate esters and amides, from trithione.[1] All of the compounds described showed a prominent band or series of bands in the infrared between 1660 and 1570 cm^{-1}. This strong absorption was assigned to the polarized double bond which conjugates the electron-donating nitrogen and the electron-accepting sulfur (A ↔ B). If structure



A

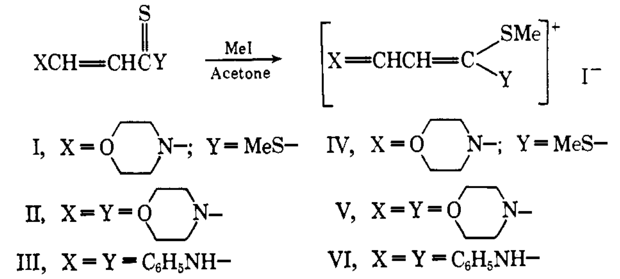


B

B makes a significant contribution to the ground-state structure, then nucleophilic displacement reactions by sulfur would be expected.

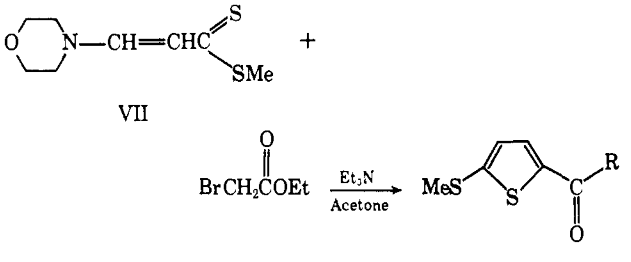
In testing this hypothesis, a new synthesis for substituted thiophenes was discovered, which makes them readily available in two steps from trithione.

When the aminothioacrylates I, II, and III were dissolved in acetone and treated with methyl iodide, there was a rapid separation of colored crystalline solids in high yields. These products proved to have the composition of starting material plus methyl iodide and were formulated as the thionium salts, IV, V, and VI. The structure proof for these compounds rests on the correct microanalytic result and infrared and nmr spectra.

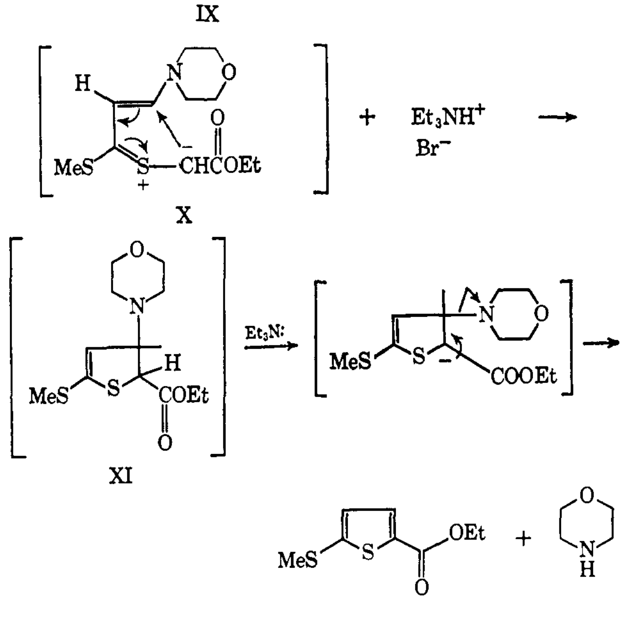


The reaction, when extended to α-halocarbonyl compounds, led to 2,5-disubstituted thiophenes rather than the expected salt. The reaction was most simply run by dissolving the thioacrylate compound in acetone and adding excess α-halocarbonyl compound followed by excess triethylamine. A very simple work-up procedure gives the substituted thiophene in good to excellent yields.

For example, from methyl 3-morpholinodithioacrylate (VII) and ethyl α-bromoacetate, the 2-methylthio-5-carboethoxythiophene (VIII, R = OEt) was isolated in almost quantitative yield. Four by-products were isolated and identified: triethylammonium hydrobromide, ethyl (α-triethylammonium)acetate bromide, ethyl (α-morpholino)acetate, and morpholine. The reaction can be run in the absence of triethylamine, but in this case the yield of substituted thiophene was substantially lower.



The reaction is a general one with α-halocarbonyl compounds. The thioacrylate ester most commonly used was compound VII, but is certainly not limited to it, as other esters work as well. Some of the thiophene derivatives prepared from methyl 3-morpholinodithioacrylate are: VIII, R = CH$_3$, 70% yield; VIII, R = C$_6$H$_5$, 70% yield; and VIII, R = C$_6$H$_4$-*p*-NO$_2$, 83% yield. The 3-morpholinothioacrylomorpholide (II) reacts readily with α-bromo-*p*-nitroacetophenone to give 2-morpholino-5-*p*-nitrobenzoylthiophene (70%



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[1] E. J. Smutny, W. V. Turner, E. D. Morgan, and R. Robinson, *Tetrahedron*, **23**, 3785 (1967).