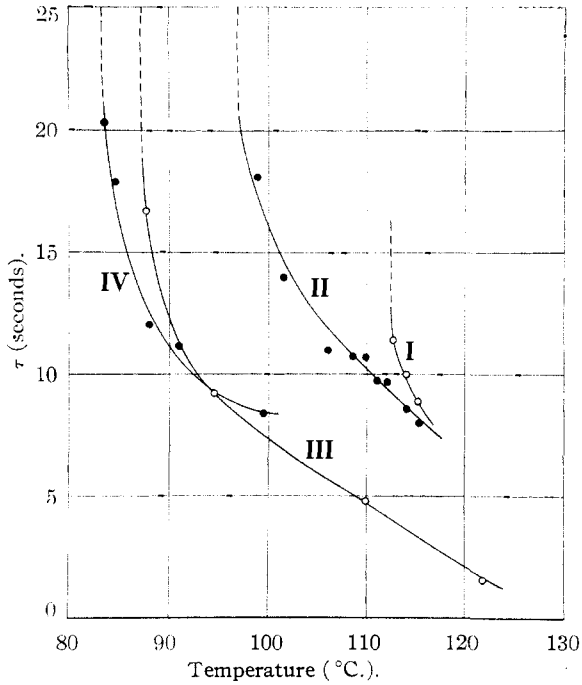

Fig. 2.—Variation of induction time with temperature: curve I, flask I, p = 32 mm.; II, flask II, 32 mm.; III, flask IV, 101 mm.; IV, flask IV, 80 mm.

satisfactory. We believe that these data can be satisfactorily interpreted through consideration of the basic processes of chain propagation. This will be considered in future publications when more data are available.

The present work represents the initial efforts of a more extensive study of the CS_2–O_2 system, which will include spectroscopic as well as ignition data.

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The Irradiation of Crystalline Vitamin B_{12} with Neutrons

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The widespread interest in the biological role of vitamin B_{12} makes its availability in radioactive form for tracer studies highly desirable. Microbiological synthesis of vitamin B_{12} labeled with Co-60[1-3] and P-32[3] has been described. However, isolation of substantial quantities of crystalline vitamin B_{12} from fermentation broths is a fairly tedious operation, and an easier route to the labeled vitamin would be desirable.

A recent communication[4] has claimed the formation of vitamin B_{12} labeled with Co-60 by the irradiation of vitamin B_{12} with thermal neutrons. Since the completion of our work another report[5] has appeared, describing the results of experiments similar to those of Anderson and Delabarre.[4] Such a procedure, if successful, would offer an attractive alternate route to the preparation of the labeled vitamin even if the specific activities obtained were not as high as those obtainable by microbiological synthesis.

In an effort to duplicate this method of preparation of Co-60 labeled vitamin B_{12} we have had a sample of the crystalline vitamin irradiated with neutrons. Our results, which differ from those published,[4,5] are based on the use of countercurrent distribution for the establishment of the identity of the Co-60 labeled material.

Experimental

Fifty milligrams of anhydrous crystalline vitamin B_{12}, sealed in an evacuated quartz vial, was irradiated for 19 days in the Brookhaven National Laboratory reactor through the courtesy of Dr. R. C. Anderson, Chemistry Department, Brookhaven National Laboratory, Upton, Long Island, New York. The irradiation was carried out at a temperature of 30–35° in a special "cold-hole" in the Brookhaven pile at a flux density of 2.7×10^{12} neutrons/cm.^{2}/sec. The exact energy spectrum of the irradiation flux was not known; besides the predominantly thermal neutron flux there were present also undetermined epithermal neutron and gamma radiation fluxes.

After irradiation the quartz vial was allowed to stand for two months to allow the decay of short-lived isotopes. The vial was then opened and the contents dissolved in water. Bioassays of an aliquot of this solution indicated that about one-third of the original biological activity remained. Another portion of the solution, when tested for Co^{++} ions using dithizone in chloroform, and α-nitroso-β-naphthol, gave negative results.

The irradiated material was purified by extraction into benzyl alcohol, after formation of the dicyanide complex, according to the procedure of Rudkin and Taylor.[6] Water was added to the benzyl alcohol extract and the vitamin B_{12} then transferred to the aqueous phase by the addition of chloroform. After decomposition of the dicyanide complex, the aqueous layer was then evaporated to a small volume and vitamin B_{12} crystallized by the addition of acetone.[5] Recrystallization was also carried out from aqueous acetone. Twenty-one milligrams of crystalline material was obtained representing 42% of the weight of the starting material. Five milligrams of this material was used in a countercurrent distribution with the system benzyl alcohol–water.[1] Each tube of the distribution was assayed for biological activity and for radioactivity.

Beta radioactivity was determined with a flow counter operating in the Geiger region; gamma radioactivity was determined with a scintillation counter.

All paper chromatograms were carried out on Whatman #1 paper. The two-dimensional chromatogram of the crystals isolated after irradiation is shown in Fig. 1. The vitamin B_{12} spot, though devoid of radioactivity, is still close to the point of application of the sample at the origin of the chromatogram. In the hope that a longer development time would result in a greater separation of the vitamin B_{12} from the other materials, a unidimensional chromatogram was carried out in which development was continued until the vitamin B_{12} spot had moved 14 cm. from the origin. An autoradiograph of this paper was made, with the results shown in Fig. 2. There are dense areas of radioactivity at the origin and at distances of 1.5, 5 and 6.7 cm. from the origin.

Countercurrent distribution of the purified crystallized sample of vitamin B_{12} was also employed to characterize the material isolated from the irradiated crystals. If Co-60 labeled vitamin B_{12} were present, the distribution of radioactivity/tube should parallel the distribution of bioactivity/

(1) L. Chaiet, C. Rosenblum and D. T. Woodbury, *Science*, **111**, 601 (1950).

(2) C. Rosenblum and D. T. Woodbury, *ibid.*, **113**, 215 (1951).

(3) E. L. Smith, D. J. D. Hackenshull and A. R. J. Quilter, *Biochem. J.*, **52**, 387 (1952).

(4) R. C. Anderson and Y. Delabarre, THIS JOURNAL, **73**, 4051 (1951).

(5) E. L. Smith, *Biochem. J.*, **52**, 384 (1952).

(6) C. O. Rudkin and R. J. Taylor, *Anal. Chem.*, **24**, 1155 (1952).

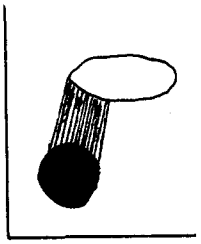

Fig. 1.—Drawing of autoradiograph from two-dimensional paper chromatogram of crystalline vitamin B_{12} isolated from irradiated material. Most of the radioactivity is at the origin with some activity extending to B_{12} area. Elliptical area, which held red color of vitamin B_{12}, was devoid of radioactivity. Horizontal development was with *s*-butanol–water, vertical development, benzyl alcohol–water.

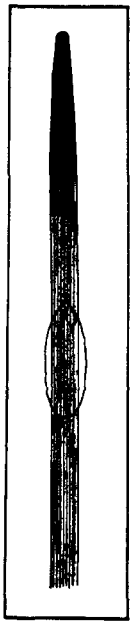

Fig. 2.—Drawing of autoradiograph of unidimensional descending paper chromatogram of crystalline vitamin B_{12} isolated from irradiated material. Four separate radioactive components are evident, as well as radioactivity extending to the solvent front. Red color of B_{12} is enclosed within elliptical area. Developing agent was *s*-butanol–water.

tube. The result of a countercurrent distribution using the benzyl alcohol–water system, is shown in Fig. 3. The distribution also demonstrates the presence of hydrophilic materials of relatively high specific radioactivity. Such high specific activity materials, present in low concentration,[7] could be "carried" by vitamin B_{12} upon repeated crystallization. The ready separation in the distribution of such materials from vitamin B_{12} is not unexpected, since compounds as closely related as vitamin B_{12} and vitamin B_{12a} can easily be resolved with this technique. Vitamin B_{12a}, in which the cyano group of vitamin B_{12} has been replaced by hydroxyl, has a distribution coefficient in the benzyl alcohol–water system of 8, while the distribution coefficient of vitamin B_{12} in the same system is 1.2.[8]

During the isolation and purification of the irradiated material, numerous small fractions were obtained that possessed both biological activity and radioactivity. Table I shows the distribution of radioactivity and biological activity among the major fractions during the isolation of the irradiated crystals.

TABLE I

DISTRIBUTION OF RADIOACTIVITY AND BIOACTIVITY AMONG MAJOR FRACTIONS DURING ISOLATION OF IRRADIATED CRYSTALS

Fraction	Radioactivity Total c./min.	%	Bioactivity Total	%
Original aqueous	1.2×10^8	100	18,000	100
Aqueous (after extraction of original aqueous with benzyl alcohol)	7.4×10^7	62	40	0.2
Benzyl alcohol (after addition of chloroform and extraction with water)	7.3×10^5	0.6	300	1.6
Vitamin B_{12} crystals	3.5×10^6	2.9	12,000	65

Discussion

Irradiation of vitamin B_{12} with thermal neutrons should give atoms of Co-60, resulting from an n,γ-reaction, with recoil energies as high as several

(7) The contents of tube O of the distribution were subjected to ultraviolet absorption analysis. No spectrum characteristic of vitamin B_{12}, or any strongly absorbing material, could be found.

(8) C. Rosenblum and D. T. Woodbury, *J. Am. Pharm. Assoc. Sci. Ed.*, **41**, 368 (1952).

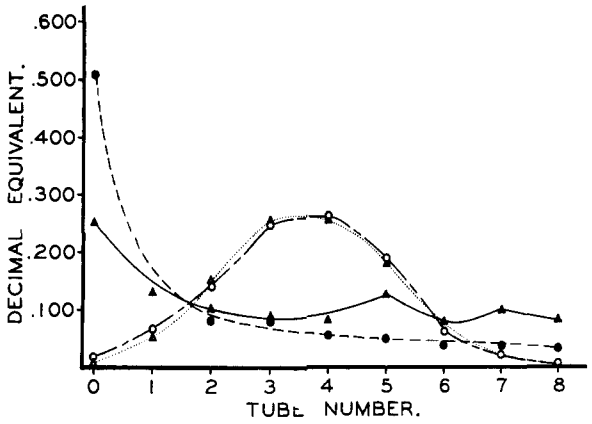

Fig. 3.—Craig countercurrent distribution on 5-mg. sample of irradiated vitamin B_{12} crystals, using the benzyl alcohol–water system: theoretical curve for $K = 1.2$, ...; biological activity curve, — — — —; gamma radioactivity, ——; beta radioactivity, – – – –.

hundred electron volts.[9] Such energies are far in excess of that required for the breaking of ordinary chemical bonds, and it would be expected that cobalt atoms, so activated, would be split from the vitamin B_{12} molecule. If some of the gamma quanta emitted during the n,γ-process were of low enough energy, some of the cobalt-60 might conceivably be retained intact within the vitamin B_{12} molecule. However, our results indicate that no detectable amounts of Co-60 labeled vitamin B_{12} were produced under our irradiation conditions.

These findings, which differ so markedly from those reported by others,[4,5] suggest that experimental conditions in studies on retention of radioactivity in complex molecules, especially those possessing specific biological activity, may require more precise definition than has been the case heretofore. The irradiation of vitamin B_{12} performed by Smith was described as being carried out with thermal neutrons in a nuclear reactor; no specification was made concerning whether or not the irradiation done by Anderson and Delabarre was carried out in a thermal column or in a nuclear reactor. It may well be that the energy spectrum of the irradiating particles was different in each case.

Although epithermal neutrons and gamma radiation were present under our experimental conditions, it would not appear likely that these contributed greatly to the destruction of the vitamin B_{12} during irradiation. Anderson and Delabarre[4] reported about 15–20% of their material had been destroyed after an irradiation cycle of seven days. Assuming a uniform rate of destruction, about 55% of their material would remain after an irradiation cycle of 19 days. Our material, irradiated for 19 days, gave a recovery of about 40% which, in view of the errors in biological assay, does not appear to be significantly different.

The two earlier reports on the irradiation of vitamin B_{12} with neutrons described the use of chromatographic techniques,[4] and the preparation of derivatives without change in specific activity,[5]

(9) R. R. Edwards and T. H. Davies, *Nucleonics*, **2**, 44 (No. 6) (1948).

for the identification of the irradiated material. While in most cases these criteria may be taken as highly indicative of the identity of two materials, the irradiation of a substance such as vitamin B_{12} warrants the application of as many criteria of identity as possible. It is conceivable that complex cobalt compounds, very closely related to vitamin B_{12}, would exhibit similar behavior on chromatography. Furthermore, the possibility must not be overlooked that these compounds might also be capable of undergoing the same exchange of anions as vitamin B_{12} when different cobalamins are made from the parent compounds. Even constant specific activity might, in this case, be misleading, especially since the desired product is present in a very small amount.

Countercurrent distribution, which can so easily effect a separation of two cobalamins as closely related as vitamin B_{12} and vitamin B_{12a}, provides an excellent additional criterion for the identity of the irradiated material. Cobalt-60 labeled vitamin B_{12}, whether prepared by neutron bombardment or by microbiological synthesis, should exhibit the same ratio of radioactivity to biological activity for each tube of a countercurrent distribution. This criterion has so far been met only in the case of microbiological synthesis.[1]

In our work the results of paper chromatography were in themselves enough to question the presence of appreciable amounts of the Co-60 labeled vitamin. The countercurrent distribution merely provided additional support for that conclusion. The results of Anderson and Delabarre[4] and Smith[5] do suggest the possibility that the Co-60 labeled vitamin had actually been prepared by neutron bombardment. However, the lack of information of the behavior of their material in a countercurrent distribution must, in view of the specificity of this technique, still leave open the claim for the preparation of Co-60 labeled vitamin B_{12} by direct neutron bombardment.

The paucity of information on the retention of induced radioactivity as a function of the energy spectrum of the neutrons used for irradiation, makes difficult an exact interpretation of the reasons for the differences between our results and those reported by others.[4,5] It is hoped that further study of these variables will lead to a better understanding of the changes resulting in complex compounds under neutron irradiation.

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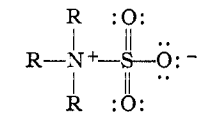
Rotations within Crystalline Trimethylamine Sulfur Trioxide

BY HEROSHI SHIMIZU AND DAN MCLACHLAN, JR.

RECEIVED APRIL 27, 1953

Although compounds of the tertiary amines with sulfur trioxide were prepared[1] in 1883 and have since been put to use in the dye industry,[2] there is still some doubt about their structure in the crystalline state.

This paper is concerned with the structure of the single compound, trimethylamine sulfur trioxide. A selection of the proper chemical structure from the four possible postulated structures of Burg[3] has been made possible by the careful work of Lecher and Hardy[4] on the basis of their own experiments, the work of other investigators,[5] the "Adjacent charge rule" of Pauling,[6,7] and spectroscopic and X-ray data of Gare and McLachlan (unpublished). Lecher and Hardy have chosen the chemical form



The present investigation needs then only to cover the possible configurations of this type of molecule in the crystal. The four possibilities were considered: 1, the R groups and O groups stationary; 2, the R groups and O groups rotating; 3, the R groups stationary and the O groups rotating; 4, the R groups rotating and the O groups stationary.

Experimental

The samples of the trimethylamine sulfur trioxide were furnished by the American Cyanamid Company through the courtesy of Dr. H. Z. Lecher. The crystals were colorless, acicular, about 5 mm. long and less than 1 sq. mm. in cross section.

Rotation pictures were taken with the crystal rotating about the longest growth axis (which proved to be the hexagonal c-axis) using molybdenum and also copper radiation. Then using copper radiation, Weissenberg X-ray pictures were taken to get the zero, first, second and third layer line pictures from which the unit cell dimensions and systematic extinctions could be determined. Using hexagonal indices, it was found that the non-extinctions were restricted to those indices which satisfy the relationship $(-h + k + 1) = 3n$ indicating a rhombohedral cell. The unit cell dimensions are: on the hexagonal lattice, $a = 9.22$ Å., $c = 6.2$ Å.; or on the rhombohedral lattice, $a = 5.71$ Å. and $\alpha = 107°\ 40'$. The number of molecules per unit cell is three for the hexagonal cell which is equivalent to one in the rhombohedral cell. The calculated density is 1.52 g./cm.^{3} and the molecular volume is 152 Å.^{3}. The space group is $C_{3v}^5 = R3m$.

The intensities of the zero layer line X-ray diffraction maxima were obtained from the X-ray films using multiple film technique (three films) and visual comparison.

Calculation of the Structure.—The intensities were corrected by the Lorentz and polarization factors[8] to get the "observed" structure factors, $|F_{hk0}|$. Since the phases associated with F_{hkl} values cannot be determined from X-ray data, a Fourier computation of the electronic densities, $\rho(xyz)$, could not be made immediately. To gain some knowledge of the structure, the trial method was used wherein the F_{hkl} values are calculated from various postulated structures and compared with the measured $|F_{hkl}|$ values. The phases are ob-

(1) F. Beilstein and E. Weigand, *Ber.*, **16**, 1967 (1883).

(2) H. Z. Lecher, M. Scalera and C. T. Lester, U. S. Patent 2,403,226 (1946). See also U. S. Patents 2,402,647 (1946) and 2,396,582 (1946).

(3) A. B. Burg, THIS JOURNAL, **65**, 1629 (1943).

(4) H. Z. Lecher and W. B. Hardy, *ibid.*, **70**, 3789 (1948).

(5) For earlier controversial work see M. Delepine and R. Demars, *Bull. Sci. Pharmacol.*, **30**, 577 (1923); M. Auerbach and R. Wolffenstein, *Ber.*, **32**, 2507 (1899); and L. Mamlock and R. Wolffenstein, *Ber.*, **34**, 2499 (1901).

(6) L. Pauling and L. O. Brockway, THIS JOURNAL, **59**, 13, 17 (1937).

(7) L. Pauling, *J. Phys. Chem.*, **56**, 361 (1952).

(8) M. J. Buerger and G. E. Klein, *J. Applied Phys.*, **17**, 285 (1946).