the reaction of an intermediate as in the case of carbon tetraiodide. At 150°, the formation of guanidine apparently reaches equilibrium at a guanidine yield of 83%. If it is assumed that the quantity of carbon tetrabromide that is converted to bromoform (presumably in a manner analogous to that suggested above for iodoform formation) is negligible, and the solution volume is taken to be 40 ml., the data for rate of ammonolysis at 125° are well represented by

$$\ln \frac{C_{\mathrm{CBr_4}}}{(C_{\mathrm{CBr_4}})_0} = -4.4 \times 10^{-4}(C_{\mathrm{Br^-}})t \qquad (2)$$

Values calculated on the basis of equation 2 are in good agreement with the experimental data for ammonolysis up to about 55% conversion; even at 70% conversion, deviation of the calculated from the experimental curve is still not great.

Since Fig. 4 shows that, if a carbon-containing intermediate were involved in this reaction, its steady state concentration would correspond to a maximum of the order of 10% of the total carbon present, hence, the possibility of isolating such an intermediate would be remote. With the single exception of the identification of bromoform as a product of reaction over relatively short time intervals, all other experiments designed to detect intermediates gave either wholly negative results or were inconclusive.

The shape of the initial portion of the curve shown in Fig. 4 suggests that the ammonolysis of carbon tetrabromide is autocatalyzed. This was confirmed by demonstrating a marked increased in initial rate upon addition of ammonium bromide as well as bromides not involving ammonium ion. The relatively greater effectiveness of sodium and potassium bromides (Table II) is probably attributable to their greater solubility in liquid ammonia.

The rate of reaction between carbon tetrachloride and ammonia is appreciable only at temperatures ≧300°. As in the case of the tetrabromide, the controlling rate is that of ammonolysis (Fig. 5). If concentrations are based upon the entire volume of the reaction tube, it may be shown that the rate data for reactions at 300° may be expressed as

$$\ln \frac{C_{\mathrm{CCl_4}}}{(C_{\mathrm{CCl_4}})_0} = -5.3 \times 10^{-6}(C_{\mathrm{Cl^-}})t \qquad (3)$$

A comparison of observed and calculated rates of ammonolysis shows excellent agreement up to about 60% ammonolysis; thereafter, a marked deviation between the calculated and observed values reflects the experimentally observed approach to equilibrium.

The data included in Table III were obtained in an unsuccessful effort to duplicate the results reported by Stähler to the effect that a 35–40% conversion of carbon tetrachloride to guanidine is obtained from the reaction with ammonia at 140° through the use of copper and iodine as the catalyst. Our results show that copper alone is devoid of catalytic activity while copper and iodine together result in only an 8% yield of guanidine; this is scarcely significant since the same yield was obtained using iron(II) oxide. With iodine in the absence of copper, the yield was 13%, but this is likely attributable to the formation of iodide ion by the interaction of iodine and ammonia. This is reasonable since it was found that iodide ion added in the form of ammonium iodide was more than three times as effective as chloride ion (at the same molar concentration) added as ammonium chloride. As shown in Table IV, the maximum effect of ammonium iodide is realized when this salt and carbon tetrachloride are present in a 1:1 mole ratio.

AUSTIN, TEXAS

[CONTRIBUTION FROM THE DIVISION OF PHARMACOLOGY, DEPARTMENT OF RADIATION BIOLOGY, SCHOOL OF MEDICINE AND DENTISTRY, UNIVERSITY OF ROCHESTER]

An Infrared Study of the Nature of Bone Carbonate[1a]

BY A. L. UNDERWOOD,[1b] T. Y. TORIBARA AND W. F. NEUMAN

RECEIVED SEPTEMBER 13, 1954

Although it has been known for many years that bone mineral and related substances contain carbon dioxide, direct evidence regarding its state in these solids has been lacking. A study of the infrared spectra of pure carbonates and bicarbonates between 11 and 12μ has confirmed the possibility of distinguishing bicarbonate from carbonate. The application of this information to the spectra of apatite, and of bone and other calcified tissues has shown clearly that the carbon dioxide is present as carbonate to the complete exclusion of bicarbonate.

Carbon dioxide is a major constituent of bone mineral, approximately 5% by weight. Its state in the solid has an important bearing on questions concerning the nature of the crystalline lattice and the physiological availability of what could be a tremendous alkali reserve. None of the several theories concerning the nature of the carbon dioxide of bone has been experimentally confirmed or refuted. Rather, there exists an array of suggestions: (a) that the carbon dioxide represents entrapped alkali bicarbonate[2]; (b) that carbon dioxide as carbonate substitutes isomorphically for phosphate ions in the crystal[3]; (c) that much of the carbon dioxide is surface-bound by adsorptive forces[4]; (d) that carbon dioxide is, in part, at least, surface-bound by an ion-exchange process displacing phosphate

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(1) (a) This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York. (b) Department of Chemistry, Emory University, Georgia.

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ions[5]; and (e) that carbonate displaces hydroxyl ions in the hydroxy-apatite lattice.[6]

Although infrared absorption spectroscopy has found its widest application in organic chemistry, a few studies of the infrared spectra of inorganic ions have been made. The most extensive catalog of such spectra has been furnished by Miller and Wilkins.[7] Included among these, are data which suggest the possibility of distinguishing bicarbonate and carbonate. It seemed that such a study might clarify, in part, the questions concerning the nature of the carbon dioxide of bone.

In most of the work reported heretofore, samples have been prepared for infrared examination by mulling in Nujol. Recently, a very useful technique of preparing infrared specimens by mixing the samples with potassium bromide and pressing transparent discs has been developed.[8,9] The utility of this method, especially for biological work, has been greatly extended by an adaptation to smaller samples.[10] Using this microadaptation of the potassium bromide pelleting technique, an infrared study of pure carbonates and bicarbonates and of apatite and bone samples has been possible which clearly shows that apatite and bone contain carbonate to the complete exclusion of bicarbonate.

Experimental

All of the infrared spectra were obtained with a Beckman Model IR 2 spectrophotometer, equipped with a beam condensing system described elsewhere[11] for the accommodation of small potassium bromide pellets. In general, 10 mg. of pure bicarbonate and carbonate salts (or 25 mg. in the case of apatite and bone samples) was ground and mixed with 1.5 g. of potassium bromide (reagent grade, previously ground to pass 200 mesh and dried at 110° overnight). The pellet was then pressed, using 35 mg. of the mixture, by means of an hydraulic press and a microdie similar to that of Anderson and Woodall.[10] The diameter of the pellet was 6.25 mm. and the force used in its preparation was about 3000 lb. The spectrum was scanned from 11 to 12.5 μ, using the strip recorder and the lowest scanning speed, with a slit width of 1.25 mm. This particular wave length region was selected for study because of the absence of phosphate interference, and because the carbonate and bicarbonate bands are better separated than is the case in other regions of carbonate absorption. The instrument was operated manually to measure more accurately the wave lengths of the absorption maxima which are mentioned in the text below.

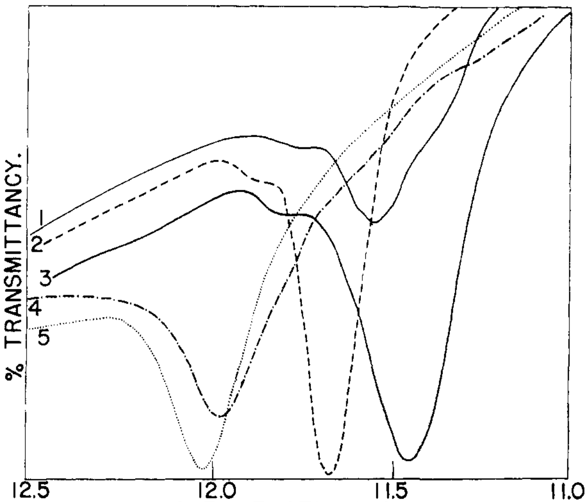

Fig. 1.—Spectra of pure carbonates and bicarbonates: 1, Na_2CO_3; 2, $SrCO_3$; 3, $CaCO_3$; 4, $NaHCO_3$; 5, $KHCO_3$.

Results

Simple Carbonate and Bicarbonate Salts.—In Fig. 1 are presented the spectra of several metal carbonates and bicarbonates. The effect of the cations in shifting the wave length of the carbonate absorption band was found in this study to be similar to that recorded by other workers.[7,12] The following wave lengths were observed: $CaCO_3$, 11.46 μ; $SrCO_3$, 11.65 μ; $BaCO_3$, 11.65 μ; Na_2CO_3, 11.55 μ; and $K_2CO_3 \cdot 1^1/_2\ H_2O$, 11.38 μ. The sodium bicarbonate band occurs at 11.91 μ. It was not possible to study the effects of cations on the bicarbonate band because the pure compounds are not available. Thus, at first glance, the possibility appears of confusing carbonate and bicarbonate absorption bands because of uncertainties in the effects of the cations. The principal cation in the apatite and bone samples is calcium. Although a spectrum of pure "calcium bicarbonate" is not at hand, there is confidence that the bands in bone and apatite which are here ascribed to carbonate are correctly identified because, as noted below, the wave length of maximum absorption is very nearly the same as in pure calcium carbonate.

Apatite.—Figure 2 shows the spectrum of a sample of apatite which had previously been well-characterized and upon which numerous other studies had been performed in this Laboratory.[13] This apatite is known from previous analyses to contain about 0.8% carbon dioxide. So far as the infrared data are concerned, it is clear that this takes the form of calcium carbonate: the carbonate

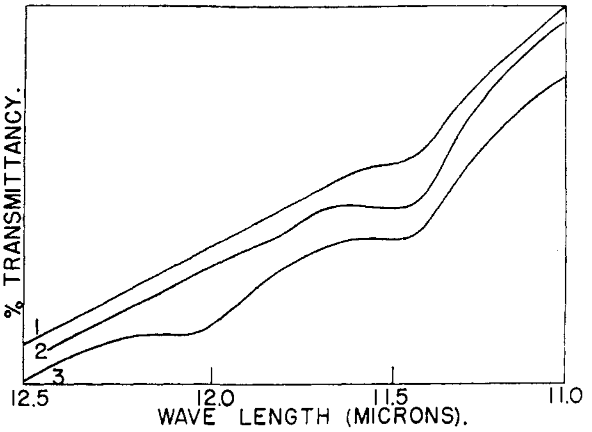

Fig. 2.—Spectra of apatite samples: 1, apatite; 2, apatite equilibrated with bicarbonate and centrifuged; 3, apatite ground dry with $KHCO_3$.

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band exhibits its maximum absorption at very nearly the same wave length (11.48 μ) as does pure calcium carbonate (11.46 μ). Also shown in Fig. 2 is the spectrum of a sample of the same apatite after equilibration with a bicarbonate solution at *p*H 7.5 followed by centrifugation at high speed according to a procedure reported elsewhere.[14] It is apparent from the spectrum that during the equilibration bicarbonate was transformed into carbonate before assuming its final state in the apatite.

It appears reasonable to postulate the over-all reaction to account for the appearance of carbonate

$$\underset{\text{(soln.)}}{HCO_3^-} + \underset{\text{(solid)}}{PO_4^{\equiv}} = \underset{\text{(solid)}}{CO_3^{=}} + \underset{\text{(soln.)}}{HPO_4^{=}}$$

in the apatite treated with bicarbonate solution. As shown by the pK_a values for bicarbonate (*ca.* 10.2) and monohydrogen phosphate (*ca.* 12.4), bicarbonate is a stronger acid than monohydrogen phosphate; thus the reaction would be expected to proceed toward the right as written provided the exchange between solid and solution can occur. Such an exchange appears reasonable on the basis of other studies.[5] It is necessary that the apatite be suspended in an aqueous phase to effect this transformation of bicarbonate to carbonate: a mixture of dry apatite and potassium bicarbonate yielded a spectrum in which the apatite carbonate absorption band was not enhanced and the bicarbonate peak was also present; but when the mixture was moistened and allowed to stand overnight, the bicarbonate band disappeared and the carbonate absorption was enhanced.

Calcified Tissues.—Spectra have been obtained with a large number of samples of bone, dentine and enamel, ranging from the fresh materials through variously treated preparations. In Fig. 3 a few of the curves are reproduced as examples of the fact that the calcium carbonate absorption band is prominent in all the specimens except those which had been treated at high temperatures and where, accordingly, no carbonate is anticipated. The glycol-ashed samples exhibit the largest absorption bands, since, in this treatment, carbon dioxide of organic origin is converted into carbonate.

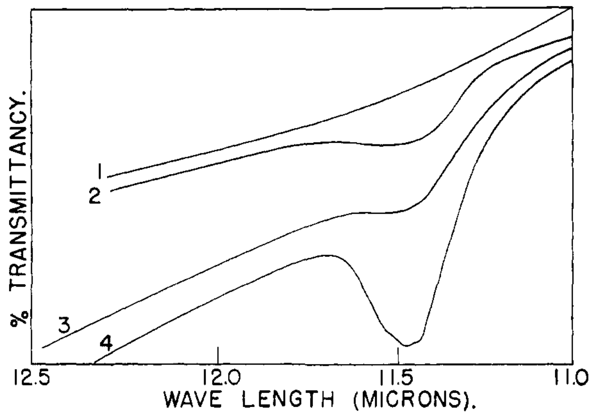

Fig. 3.—Spectra of calcified tissues: 1, enamel, ashed above 750°; 2, fresh enamel; 3, veal shaft, unashed, fat-extracted; 4, glycol-ashed rabbit bone.

The results summarized above clearly indicate that the carbon dioxide of bone and related materials is present entirely as carbonate. While this is reasonable, and, indeed, has been tacitly assumed by many workers, the infrared evidence appears to be the first direct verification of the idea. Preliminary studies indicate that an infrared method for the quantitative determination of bone carbonate will prove feasible.

ROCHESTER, NEW YORK

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[CONTRIBUTION FROM THE JAMES FORRESTAL RESEARCH CENTER, PRINCETON UNIVERSITY]

The Vibrational Spectrum of Methyl Nitrate[1]

BY J. C. D. BRAND AND T. M. CAWTHON[2]

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The infrared absorption of methyl nitrate in the vapor and liquid phase has been measured in the range 2–15μ and frequencies have been assigned to all the fundamental vibrations except the two torsional modes. The characteristic frequencies of the $-ONO_2$ group are at 1672 (vs), 1287 (s), 854 (s), 759 (m), 657 (m), and 578 (m) cm.$^{-1}$. Comparison of the statistical and thermal entropies indicates that the barriers hindering internal rotation are small, and the infrared band contours and Raman depolarization factors are interpreted assuming free rotation about the O–N bond.

Introduction

The Raman spectrum of methyl nitrate has been recorded by several observers.[3] Apart from a small number of weak or doubtful bands the measurements of frequency and depolarization factors[3b,c] are in good agreement and the main aspects of the spectrum are firmly established. The infrared absorption has been investigated for both the liquid[3c,4] and vapor[5] phase, but the resolving power was less than is now available and band contours of the vapor spectrum were not determined.

The structural evidence is not complete. The only nitrate ester examined by X-ray methods is pentaerythritol tetranitrate,[6] wherein each nitrate

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[1] This research was supported by the United States Air Force under Contract No. AF33(038)-23976 monitored by the Office of Scientific Research.

[2] Allied Chemical and Dye Fellow, 1953–1954.

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