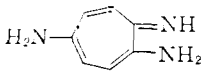
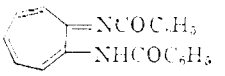


III



IV

The 5-amino derivative III was obtained by nitrosation of tropolone and then oximation and reduction.[2] Very recently the dibenzoyl derivative IV of 1-amino-7-imino-1,3,5-cycloheptatriene was prepared from 1,3-diazaazulene by reaction with benzoyl chloride.[3] Attempted hydrolysis of IV yielded 2-phenyl-1,3-diazaazulene with either acidic or basic catalysts.

The aminoimines I were obtained in 30–80% yield by reaction of the tetrafluorocycloheptadienes with primary amines and with ammonia.

I (R = H): (hemihydrate), 30% yield of yellow crystals from ether, m.p. 112–113°. *Anal.* Calcd. for $C_7H_8N_2 \cdot 1/2H_2O$: C, 65.09; H, 7.02; N, 21.69. Found: C, 65.01; H, 6.93; N, 21.54.

I (R = H): CF_3COOH salt. *Anal.* Calcd. for $C_7H_8N_2 \cdot CF_3COOH$: N, 11.96. Found: N, 11.92.

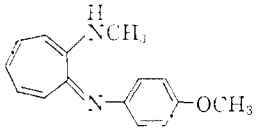
I (R = CH_3): 75% yield of yellow crystals from methanol, m.p. 66.5–67°. *Anal.* Calcd. for $C_9H_{12}N_2$: C, 72.93; H, 8.16; N, 18.91. Found: C, 73.05; H, 8.26; N, 18.92.

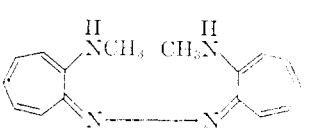
I (R = *p*-ClC_6H_4): 70% yield of red crystals, m.p. 168–170°. *Anal.* Calcd. for $C_{19}H_{14}N_2Cl_2$: C, 66.87; H, 4.13; N, 8.21. Found: C, 66.71 H, 4.22; N, 8.13.

The infrared and ultraviolet spectral data are interpreted as being consistent with the proposed structures. Furthermore, nuclear magnetic resonance studies indicated that the nitrogen atoms of the dimethylaminoimine I (R = CH_3) are identical. Thus, either the hydrogen atom is shared equally between the two nitrogen atoms or there is rapid exchange of hydrogen between the atoms.[4]

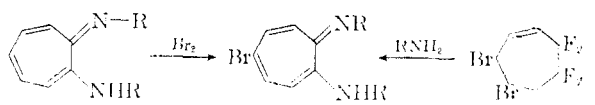
The adjacency of the nitrogen atoms was further established by formation of stable chelates with Ni^{++}, Co^{++}, and Cu^{++} ions, and by conversion of the aminoimines to tropolone.

Although stable to strong aqueous acids or bases, the dimethylaminoimine I (R = CH_3) reacted with amines in dioxane containing a catalytic amount of acetic acid to yield new aminoimines derived by displacement of the methylamino group.





Ring substitution has been effected by various electrophilic reagents. In contrast to tropolone, which gives a mixture of mono-, di- and tribromo derivatives, reaction of I (R = *p*-ClC_6H_4) with one mole of bromine gave a single isomer in 90% yield, red crystals, m.p. 181–182°. It was established indirectly that substitution had occurred at the 4-position by condensation of 3,4-dibromo-6,6,7,7-tetrafluorocycloheptene with *p*-chloroaniline to obtain a monobromoaminoimine identical with the product obtained from the bromination reaction.



The 1-amino-7-thioxo-1,3,5-cycloheptatrienes II were obtained by action of hydrogen sulfide on the aminoimines I. The N-substituted derivatives are stable compounds, soluble in dilute acids and insoluble in strong bases. Nuclear magnetic resonance studies of the nitrogen atom indicate that there is little, if any, contribution to the structure by the iminothiol tautomer.[4] Chelate derivatives were formed with Cu^{++}, Ni^{++} and Co^{++} ions.

II (R = H), 60% yield of yellow crystals from methanol, m.p. 137–138.5°. *Anal.* Calcd. for C_7H_7NS: C, 61.27; H, 5.15; N, 10.21; S, 23.37; mol. wt., 137.2. Found: C, 61.20; H, 5.15; N, 9.94; S, 23.40; mol. wt., 122.

The corresponding N-methyl and N-*p*-tolyl derivatives have been synthesized and the observed analytical data are in accord with the proposed structures.

These and other reactions of the aminoimines I and the aminothioxocycloheptatrienes II will be fully reported shortly.

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(3) I. Murata, *Bull. Chem. Soc. Japan*, **32**, 841 (1959).

(4) We are indebted to Drs. W. D. Phillips and D. B. Chesnut for this determination and interpretation.

QUANTITATIVE ASPECTS OF NUCLEATION IN CALCIUM PHOSPHATE PRECIPITATION[1]

Sir:

After many many years of a single-minded view, prompted primarily by the theories of Robison,[2] a new and different approach to the problem of biologically induced calcification has developed. Currently, it is almost universally recognized that collagen fibers possess the unique property of inducing crystals of calcium phosphate to form.[3,4] This change in viewpoint resulted primarily from a clarification of the solubility properties of bone mineral itself.[5,6] It is now clear that normal serum is highly supersaturated with respect to bone mineral *if solid phase is present* as it always is in the living animal. Bone mineral prototypes (hydroxyapatites) and dead bone powders evince widely varying dissolution products (Ca × P inorganic) which are strongly dependent upon experimental conditions, but rarely if ever are products greater than 10 (Ca × P_i in (mg.%)^2) encountered. Living bone in culture, however, will support higher products[7,8] approaching the physiological (*ca.* 20).

(1) 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.

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TABLE I

INDUCTION OF CRYSTAL FORMATION BY COLLAGEN

Determination of the point of precipitation was accomplished by techniques published earlier (Fig. 1, ref. 5). Conditions were $\mu = 0.16$; $t = 37°$; pH 7.4; time of equilibration, 3 days.

	No. expts.	Point of precipitation[a] Mean	Standard deviation	Standard deviation of mean
Control solutions	7	50	4.1	1.6
Collagen-seeded	11	20	4.7	1.4

[a] Expressed as Ca × P_i in (mg. %)².

Presumably, this phenomenon results from localized acid production by the living bone cells themselves.[8,9]

In the absence of preformed solid phase, it is the K_{sp} of $CaHPO_4 \cdot 2H_2O$ which seems to govern the stability of the aqueous calcium:phosphate system.[5] Spontaneous precipitation never has been described in solutions having products, Ca × P_i, less than about 35 (mg. %)[5] although varying degrees of metastability have been observed (depending upon conditions) with solutions having products as high as 50. These general findings are summarized in Fig. 1.

Until now the idea that collagen can function as a crystal nucleator *in vivo* has suffered from a serious quantitative defect. Crystal induction by collagen has been demonstrated in several laboratories.[5,10,11] The process has been shown to be exquisitely specific[4,10] and morphologically the early phases of crystal induction are very similar both *in vivo* and *in vitro*.[10,12] Nonetheless, until the present, these examples of crystal induction have all involved solutions in the region of metastability ($>K_{sp}$ $CaHPO_4 \cdot 2H_2O$) involving products of Ca × P_i from 35 to 50 (mg.%)².[3,5,11,13] Yet to be functional in the living animal, collagen must be shown to induce crystal formation at physiological products (*ca.* 20), a region of undersaturation with respect to solid formation.

Quite recently,[14] crystal induction has been demonstrated with demineralized, fresh dentin at products, Ca × P_i, of 20, well below the critical K_{sp} $CaHPO_4 \cdot 2H_2O$. This represented the first occasion, to our knowledge, of crystal nucleation by a non-living system under physiologically meaningful conditions. The tissue residue employed, however, undoubtedly contained substances other than collagen. Hence, the case for collagen as the nucleator, though greatly strengthened in a quantitative sense, was still not proven. In experiments just completed, a collagen[15] prepared by the method of Einbinder and Schubert[16] also has proved able to cause crystal formation in solutions of physiological concentrations of calcium and phosphate. These data are summarized in Table I.

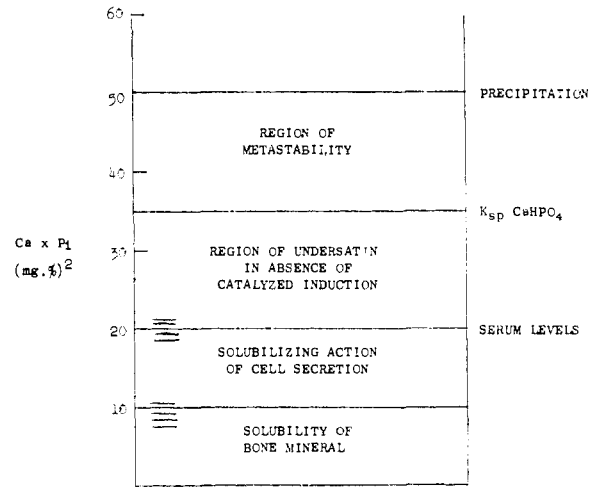

Fig. 1.—A summary of present information concerning dissolution and formation of bone mineral given in terms of the serum product, Ca × P_i, in (mg. %)².

The case for collagen now seems complete. It *can* specifically cause crystal formation under physiological conditions. *Whether* it does this *in vivo* and *how* it accomplishes this remain questions yet to be answered by further research.

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PROTON RESONANCE SPECTRUM AND STRUCTURE OF THE AZULINIUM ION

Sir:

The structure of the azulinium ion is of considerable interest in connection with the electron charge distribution in azulene itself. Protonation of azulene in acid solutions may be expected to occur at the 1 and 3 positions, which, according to molecular orbital calculations,[1] have the greatest excess π-electron charge density. It is also known that the protons at the 1 and 3 positions of azulene are readily exchanged in D_2SO_4 solutions.[2] We have now been able to confirm the structure of the azulinium ion on the basis of a high-resolution proton resonance spectrum. A previous attempt[3] to determine the structure from the proton resonance in concentrated sulfuric acid was unsuccessful due to inadequate resolution.

Figure 1a shows the proton resonance spectrum of a 7 mole % solution of pure azulene in CCl_4. A scale in c./s., referred to the resonance signal of CH_2Cl_2 (dotted line), is shown at the bottom of the figure. The assignment and analysis of the azulene proton spectrum has been described previously.[4] Shown on the same scale in Fig. 1b is the proton resonance spectrum of a 7 mole %

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(15) Upon analysis, this preparation was essentially Ca-free, certainly less than 0.1 p.p.m.

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