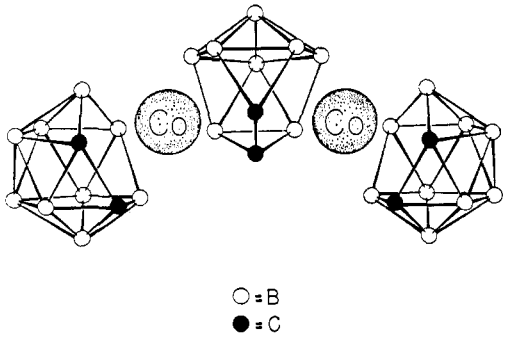

Figure 1. Skeletal structure of $[(C_2B_9H_{11})Co(C_2B_8H_{10})Co(C_2B_9H_{11})]^{2-}$.

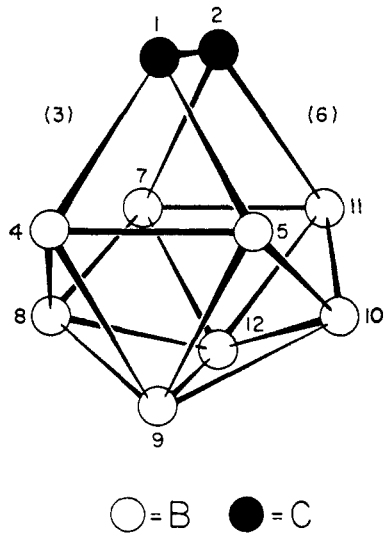

Figure 2. Skeletal structure of the (3,6)-1,2-dicarbacanastide ion.

the first example of a bidentate π-bonding ligand which is derived from a ten-particle icosahedral fragment.

We have used the reaction of $1,2\text{-}B_9C_2H_{11}^{2-}$ with transition metal hydroxides (formed *in situ* from the chlorides in hot aqueous base) as a convenient preparation of the $(B_9C_2H_{11})_2M$ systems.[1a,c] Precipitation of the orange $Rb^+[(B_9C_2H_{11})_2Co^{III}]^-$ from an aqueous solution of the ether-soluble products of such a reaction leaves a red solution, from which we have isolated in low yield (1%) a new ion (I) as the cesium salt. Yields of 15% may be obtained by treating preformed $K^+[(B_9C_2H_{11})_2Co^{III}]^-$ with 30% aqueous NaOH and excess $CoCl_2$ for several hours at 100°. The iron and nickel complexes do not give similar reactions under these conditions.

A single-crystal X-ray diffraction study[2] has now confirmed that the compound has the structure presented in Figure 1. For the novel central ligand (Figure 2) we propose the root name "canastide," from the Spanish noun for basket. Thus, the name of the central ion would be the (3,6)-1,2-dicarbacanastide-(−4) ion, and that of compound I would be bis-π-[(3)-1,2-dicarbollylcobalt]-π-(3,6)-1,2-dicarbacanastide-(−2) ion. *Anal.* Calcd for $[C_6H_{32}B_{26}Co_2]Cs_2 \cdot H_2O$: C, 9.16; H, 4.35; B, 35.73; Co, 14.92. Found: C, 9.60; H, 4.34; B, 36.47; Co, 14.63. The 60-Mc/sec ^1H nmr spectrum in acetone solution contained resonances at δ 3.8 (broad, 4 H) and 4.63 (broad, 2 H). These resonances can be assigned to the protons on the carbon atoms of the two outer and central ligands, respectively. The protons attached to the boron atoms gave resonances too broad to be observed. The 32-Mc/sec ^{11}B nmr shows a doublet at δ −21 (ppm from $BF_3 \cdot OEt_2$) of area 2, and an envelope from δ −6 to +26 of area 26 resembling the spectrum of $(B_9C_2H_{11})_2Co^-$. The infrared spectrum (Nujol mull) contained bands at 1595, 3580, and 3450 cm^{-1} due to a water of crystallization, as well as carborane CH (3000 cm^{-1}) and BH (2500 cm^{-1}) bands. Electronic spectra of the $(CH_3)_4N^+$ salt in acetonitrile revealed λ_{max} (ϵ) at 297 mμ (40,000) and 324 mμ (30,000) sh, and a long-wavelength end absorption, 800 mμ (160). Cyclic voltammetry in acetonitrile showed two reversible couples with half-wave potentials +0.95 and −1.50 V (*vs.* sce).

Presumably, a boron of one of the cages of the $(B_9C_2H_{11})_2Co^-$ complex is removed by the strong base, and the open face so formed then complexes with another cobalt atom and a $B_9C_2H_{11}^{2-}$ ion. The $B_9C_2H_{11}^{2-}$ ion must arise from the dissociation or degradation of the starting material. To confirm the availability of the $B_9C_2H_{11}^{2-}$ ion in solution, $(B_9C_2H_{11})_2Co^-$ was treated under the same conditions with nickelous chloride. Benzene was added to the cooled reaction mixture, and air was bubbled through the whole for several hours. A low yield (1–2%) of $(B_9C_2H_{11})_2Ni$,[1c] identified by infrared spectroscopy and thin layer chromatography R_f values, could be obtained from the benzene solution. The solution remaining contained a variety of products, among them, presumably, a complex similar to I, but containing one nickel and one cobalt atom, which has not been isolated.

The existence of the (3,6)-1,2-dicarbacanastide ion suggests the possible existence of a large family of new metal complexes, some of which may contain cyclic ligand–metal arrays and highly delocalized bonding electrons. An intensive investigation of this chemistry is in progress.

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(2) The authors wish to thank Professor David H. Templeton, Dr. Alan Zalkin, and Mr. D. St. Clair for these structural results received prior to publication.

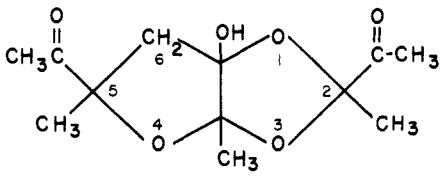
A Simple Trimerization of 2,3-Butanedione Yielding a Selective Reagent for the Modification of Arginine in Proteins

Sir:

Selective modification of amino acid side chains in proteins constitutes an important avenue for correlation

of protein structure and function. At present there is no report of a stable crystalline reagent for the chemical modification of arginine under mild conditions. α-Diketones have been used by Itano and coworkers[1] to selectively modify arginine in 0.2 *N* base while King[2] has reported a selective modification in 10 *N* HCl. The pH extremes required for these modifications appear to limit their direct applicability to proteins to special cases. Since the guanidino group of arginine exists in its resonance-stabilized protonated form over the entire pH range of protein stability, it is not surprising that modification of this functional group under mild conditions requires uncovering of unique reagents.

Experiments in our laboratory on the interaction of 2,3-butanedione (biacetyl) with guanidinium salts at pH 8.2 provided evidence that modification of the guanidinium group was occurring after a preliminary self-condensation of this diketone.[3] A reagent generated in the above manner has already been employed by Grossberg and Pressman[4] in the study of arginine at active sites of antibodies. Examination of known oligomers of biacetyl has now shown that the crystalline trimer, 2,5-diacetyl-3a,5,6,6a-tetrahydro-6a-hydroxy-2,-3a,5-trimethylfuro[2,3-*d*]-1,3-dioxole (I), first prepared by Cresswell, *et al.*,[5] is a rapid and selective reagent for modification of arginine under mild conditions. In the present communication we report a novel and convenient preparation of I and the application of this trimer to bovine plasma albumin (BPA) and bovine pancreatic ribonuclease-A (RNase).



I

Initial preparations of trimer I were accomplished by employing anion-exchange resins as the alkaline condensing agents according to the method of Cresswell, *et al.*[5] Compound I was also reported by these workers to be found occasionally in old bottles of biacetyl.[5] Accordingly, we have found that improved and reproducible yields of I can be obtained by employing alkalinized powdered glass as a condensing catalyst. In a typical preparation 196 g of biacetyl was mixed with 415 g of clean,[6] base-washed (0.1 *N* NaOH, H_2O), dry, powdered glass (E. H. Sargent and Co., SC-12300). After 5 days at room temperature with occasional mixing, the mass hardened. The product was extracted with ether and the extract was treated with magnesium sulfate. Following removal of solvent and overnight storage in the cold, 76 g (39%) of I was collected. Recrystallization from ether gave white needles, mp 112.5–114° (lit.[5,7] 73–75°), λ_{max}^{KCl} 2.86, 5.82 μ; nmr ($CDCl_3$) τ 5.52 (1 H), 7.66 (3 H), 7.75 (3 H), 8.54 (3 H), 8.60 (3 H), 8.66 (3 H), 6.74 (d, J = 13.5 cps, 1 H), 8.09 (d, J = 13.5 cps, 1 H); λ_{max}^{1mMHCl} 286 mμ (ε 84). *Anal.* Calcd for $C_{12}H_{18}O_6$: C, 55.81; H, 7.02; mol wt, 258. Found: C, 56.00; H, 7.04; mol wt (osmometer, acetone), 256.

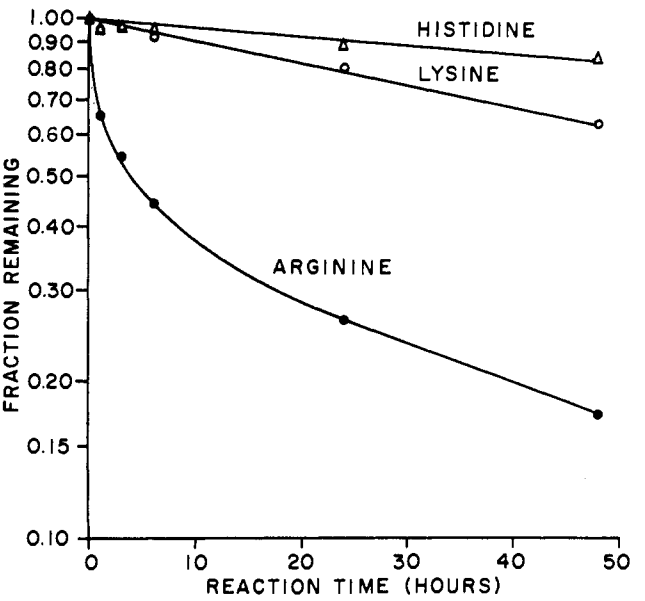


Figure 1 The loss of amino acids from bovine plasma albumin exposed to biacetyl trimer I (0.4 *M*) at 25° in pH 7.0, 0.5 *M* phosphate buffer. The combined aspartic acid, valine, and phenylalanine content of each hydrolysate was used as the basis for calculating recovery of other amino acids.

Preliminary examination of the ability of I to modify arginine was done by thin-layer chromatography on silica gel plates using ammoniacal propanol (3:7) as developing solvent and ninhydrin as the location reagent. The ability of I to modify BPA, RNase, and free amino acids in addition to arginine was examined by amino acid analysis on a Technicon Model NC-1 amino acid analyzer.

In a typical experiment bovine plasma albumin (6 mg/ml) was allowed to react with 0.4 *M* reagent in 0.5 *M*, pH 7.0 phosphate buffer at 25°. At fixed intervals samples were removed and dialyzed extensively against distilled, deionized water at 4°. The dialyzed protein samples were lyophilized, hydrolyzed (6 *N* HCl, 24 hr, 110° under vacuum), and subjected to amino acid analysis. Figure 1 shows a semilogarithmic plot of arginine, lysine, and histidine recovery against time of exposure to the reagent prior to dialysis. The presence of both exposed and buried guanidinium groups is suggested, a phenomenon well known for other protein side chains. A similar behavior was noted for RNase with the exception that no histidine loss occurred. Somewhat greater selectivity is noted at pH 6.0. Thus, after 6.0 hr at this pH, 53% of the arginine is lost from BPA while lysine loss is at the level of experimental error (3%). Modified residues are destroyed by acid hydrolysis.

Analysis of a mixture of free amino acids (1 m*M*, 0.4 *M* reagent) after 1-hr exposure to the reagent showed a

(1) H. A. Itano and A. J. Gottlieb, *Biochem. Biophys. Res. Commun.*, 12, 405 (1963); K. Toi, E. Bynum, E. Norris, and H. A. Itano, *J. Biol. Chem.*, 242, 1036 (1967).

(2) T. P. King, *Biochemistry*, 5, 3454 (1966).

(3) J. A. Yankeelov, Jr., M. Kochert, J. Page, and A. Westphal, *Fed. Proc.*, 25, 590 (1966).

(4) A. L. Grossberg and D. Pressman, *ibid.*, 26, 339 (1967); A. L. Grossberg and D. Pressman, *Biochemistry*, 7, 272 (1968).

(5) R. M. Cresswell, W. R. D. Smith, and H. C. S. Wood, *J. Chem. Soc.*, 4882 (1961).

(6) L. A. Carlson, *Clin. Chim. Acta*, 5, 528 (1960).

(7) Melting points in this report were obtained on a Fisher-Johns apparatus employing cover glasses dealkalinized with 1 *N* HCl.

97% loss in arginine, 18% loss in serine, and variable loss of methionine.[8] Losses of other susceptible amino acids (threonine, glycine, cystine, lysine, and histidine) were below 10%. Reactions of the neutral free amino acids appear to occur *via* the α-amino groups. The reagent does not modify free tyrosine at neutral pH, although low recovery of tyrosine is found in hydrolysates of modified proteins. The loss occurs during the hydrolysis step and can be eliminated by including sacrifical phenol in the hydrolysis mixture.

Chromatographic analyses of samples of free arginine treated with stoichiometric amounts of reagent show two major, ninhydrin-positive, final products. Study of the products from this and related reactions is continuing in an effort to define the reaction mechanism.

Acknowledgments. We are indebted to Dr. H. C. S. Wood for kindly supplying us with a sample of authentic I and for useful comments concerning the resin-catalyzed preparation. The authors wish to acknowledge the invaluable assistance of Mr. Malcolm Kochert and Miss Susan Weber throughout this work. This research was supported by Grants GB-2033 and GB-4731 of the National Science Foundation.

(8) Loss of methionine is apparently due to a noncovalent interaction with the reagent. The resulting complex is ninhydrin positive and appears just prior to aspartic acid.

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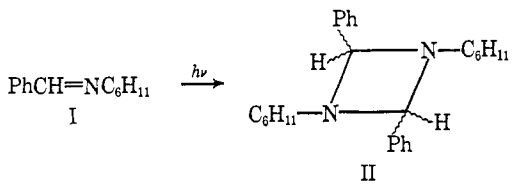
Photochemical Formation of 1,3-Diazetidines

Sir:

The multitude of reported photochemical dimerizations[1] is noteworthy for the absence among them of the formation of diazetidines from the combination of C=N containing monomers.[2] We now report the photochemical dimerization of benzaldehyde cyclohexylimine (I) to yield a stable dimer to which we have assigned the structure of N,N′-dicyclohexyl-2,4-diphenyl-1,3-diazetidine (II).



I II

The benzophenone-sensitized irradiation of ethanolic or 2-propanolic solutions of I for 24 hr at 25° with artificial or solar ultraviolet light[3] yields 34% of II. The structure of II was elucidated on the basis of its physical and chemical properties. N,N′-Dicyclohexyl-2,4-diphenyl-1,3-diazetidine is a solid, mp 143–144°. The elemental analysis[4] (*Anal.* Calcd for $C_{26}H_{34}N_2$: C, 83.36; H, 9.15; N, 7.48; mol wt, 374. Found: C, 83.38; H, 9.24; N, 7.56; mol wt, 376) indicates that it is a dimer of I. The ultraviolet spectrum (λ_{max} (dioxane) 265 mμ (ε 480)) is indicative of two isolated benzene rings. The infrared spectrum ($CHCl_3$) shows no other features than aliphatic and aromatic C–H and the presence of monosubstituted aromatic rings (3030 w, 2990 m, 2900 s, 2810 m, 1580 w, 1450 s, 720 m, and 700 s cm^{-1}). The 60-Mc nmr spectrum shows the aromatic hydrogens as a singlet at τ 2.88 (10 H), the two ring hydrogens as a singlet at τ 6.29 (2 H), and cyclohexyl hydrogens at τ 7.92 (broad, 2 H) and 8.1–9.4 (broad, 20 H), the former being assigned to the carbon atoms attached to nitrogen.

Examination of the natural abundance ^{13}C–H satellite spectrum[5] of the ring hydrogens demonstrated the satellite to be a singlet, with a half-width of 3.5 cps. This fact, together with the pyrolysis studies cited below, allows the selection of a 1,3-diazetidine over a 1,2-diazetidine; the two equivalent ring hydrogens of the latter would have doublet satellites.

The mass spectrum[6] of II is noteworthy for the ease of elimination of $C_6H_{11}N$ from the molecular ion, resulting in a peak with *m/e* 277 ($M^+ - C_6H_{11}N$) as the highest *m/e* peak. The stability of this fragment is expected in view of the stabilization allowed through two benzylic systems. A similar result is obtained with the dimer of *p*-chlorobenzaldehyde cyclohexylimine, and only a change to direct inlet at 110° revealed the expected M^+ peaks. All other fragments are in complete agreement with the structure of II. Most prominent are those due to the loss, in addition to the $C_6H_{11}N$ fragment mentioned above, of C_6H_{11}, C_6H_5C, C_6H_5CH, and a second $C_6H_{11}N$.

In view of the above-noted instability of the molecular ion, we investigated the pyrolysis of II and its more readily available *p*-chloro derivative (mp 183–184°), the infrared, ultraviolet, and mass spectral properties of which were entirely analogous to those of II. Heating of either dimer causes sublimation, but decomposition occurred only after heating in a sealed tube at 235° for 30 min. The only identifiable products from such mixtures were the dimer and the monomer, obtained in yields up to 30%, giving evidence of the wide variance in thermal stability between the molecular species and its ions.

The transient formation of a 1,2-diazetidine, proposed by Searles and Clasen[7] to account for the reported isolation of stilbenes and azobenzene derivatives from the photolysis of a substituted anil, prompted us to look for similar products in the pyrolysis mixtures; although such stilbenes would have survived the reaction conditions, they were never observed. These products would of course not be expected to form from a 1,3-diazetidine.

A surprising characteristic of II is its unusual chem-

(1) For a review see R. O. Kan, "Organic Photochemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1966.
(2) The dimerization of 2-aminopyridines is a case in point: E. C. Taylor and R. O. Kan, *J. Am. Chem. Soc.*, **85**, 776 (1963).
(3) A Hanovia 450-W immersion lamp equipped with a Pyrex filter or a Rayonet photochemical reactor with 3500-Å lamps will suffice.
(4) Spang Microanalytical Laboratory, Ann Arbor, Mich., and Galbraith Laboratories, Inc., Knoxville, Tenn.
(5) A Varian A-60 nmr spectrometer equipped with spin decoupler and computer of average transients was employed.
(6) Morgan Shaffer Corp., Quebec, Canada.
(7) S. Searles, Jr., and R. A. Clasen, *Tetrahedron Letters*, 1627 (1965).