this result was interpreted on the basis of "several reinforcing and opposing factors." As the cause of deceleration, it is pointed out further that the presence of the C(7) methylene bridge in **11** must increase the strain energy associated in the ionization, thus destabilizing the transitions tate and the resulting trishomocyclopropenyl cation intermediate.[9] Since the present system **3a** is missing such a bridge, one must seek other explanations for the slow rate of **3a** compared with **1**. If one considers the difference between angle [C(1)–C(6)–C(5)] of **3a** and angle [C(1)–C(8)–C(5)] of **1**,[10,11] it appears that the anchimeric assistance provided by the cyclopropane participation in the solvolysis of **3a** would be at least of the same order of magnitude as that estimated for **1** and very likely would be slightly more.[11] Thus, as the X-ray analysis of **3a** demonstrates the anomolous distortion of the system, the critical interplanar angle (*vide supra*) of system **1** could possibly be just as large as that of **3a** again due to the internal van der Waals interactions between C(3)–H and C(6)–H and C(7)–H of **1**.[12] Unfortunately, crystallographic data of **1** are not available. Finally, brief comments should be made on the chemical properties of **4**. As earlier noted,[4] the solvolysis rate of **4** is large (5×10^3) as compared with its homolog, *anti*-bicyclo[2.2.1]hept-2-en-7-ol ester (**10**) (*cf.* k(**3a**)/k(**1**) = 0.1). This accelerated solvolytic reaction of **4** and other observations such as the *stereoselective* addition (anti to the substituted bridge) of a methylene group (*vide supra*) and diazomethane (pyrazoline formation)[13,14] to **4** suggest that C(5) of **4** may be unsymmetrically bent toward the double bond unexpectedly and sufficiently to bring about these seemingly unusual experimental results.

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(10) P. D. Bartlett and W. P. Giddings, *J. Amer. Chem. Soc.*, **82**, 1240 (1960).

(11) C. S. Foote, *ibid.*, **86**, 1853 (1964); P. von R. Schleyer, *ibid.*, **86**, 1854 (1964). The ketone **12** corresponding to **3d** was prepared by Oppenauer oxidation and exhibited a ν_{max} at 1788 cm^{-1} (CCl_4). If the Foote–Schleyer equation is still applicable to these strained systems, then the anchimeric assistance calculated for the solvolysis of **2a** is *ca.* 500 larger than that for **1**.

(12) Obviously we are assuming that the energy required for ionization increases as the ground-state geometry of a system departs from that of the corresponding cation which is ideally stabilized by the cyclopropane participation.

(13) S. Masamune and P. Vokov, unpublished results.

(14) Also see, W. R. Roth and A. Friedrich, *Tetrahedron Lett.*, 2607 (1969).

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Mixed Charge Exchange–Chemical Ionization Reactant Gases in High-Pressure Mass Spectrometry

Sir:

Electron-impact ionization mass spectra often lack abundant ions of high mass. For this reason other methods of ionization such as chemical ionization[1,2] are gaining acceptance as complements to electron-impact ionization. Chemical ionization mass spectrometry requires a reactant gas from which reactant ions are formed by electron-impact ionization and ion–molecule reactions.[1,2] A single reactant gas is generally used in the chemical ionization source. A discussion of two-component reactant gas systems limited to mixtures of charge exchange and chemical ionization reactant gases is presented herein. For illustrative purposes the two gases selected are helium, a charge-exchange reactant gas, and water vapor, a chemical ionization reactant gas.

Helium charge-exchange mass spectra measured under chemical ionization conditions[1,2] have been reported[3,4] to be similar to electron-impact ionization mass spectra. Gaseous mixtures made up of helium and water vapor may be varied so that the mass spectrum of the substance under examination will show peaks characteristic of charge-exchange ionization, of chemical ionization, or of both. Spectra which show peaks from both modes of ionization are referred to herein as simultaneous charge exchange–chemical ionization mass spectra.[5] Mass spectra of methyl 6,10,14-trimethylpentadecanoate (**1**), the methyl ester of a naturally occurring isoprenoid fatty acid,[6] are shown in Figure 1 to illustrate changes in mass spectra with variations in reactant gas mixtures.

The mass spectra (Figure 1) were measured with a modified[7] QUAD 300 quadrupole mass spectrometer. The sample (**1**) was vaporized directly into the high-pressure ion source held at *ca.* 0.5 Torr and 200°. The helium flow rate was 1.2 ml/min and the water content of the gaseous mixture was varied from 0 (Figure 1a and b) to 6 (Figure 1c)[8] to 13%[8] (Figure 1d). The sample (**1**) concentration was the same for all spectra.

Figure 1a shows the mass spectrum of **1** taken with the electron-impact ion source in series[7] with the high-pressure ion source. The helium charge-exchange mass spectrum of **1** (Figure 1b) is comparable although not identical. Both electron-impact and helium charge-exchange mass spectra of **1** show, for example, characteristic peaks at m/e 74 and 222 and a molecular ion peak (m/e 298) of such low abundance as to be hardly detectable. By contrast, the simultaneous mass spectrum (Figure 1c) shows an abundant $(M + 1)^+$ at m/e 299, one of the two most abundant ions in the chemical ionization mass spectrum (Figure 1d), which clearly points to the molecular weight (298) of **1** while retaining the peaks (*e.g.*, m/e 74 and 222) characteristic of electron-

(1) M. S. B. Munson and F. H. Field, *J. Amer. Chem. Soc.*, **88**, 2621 (1966).

(2) For recent reviews, see (a) B. Munson, *Anal. Chem.*, **43**, 28A (1971); (b) G. P. Arsenault in "Biochemical Applications of Mass Spectrometry," G. R. Waller, Ed., Wiley-Interscience, New York, N. Y., 1972, pp 817–832.

(3) D. M. Schoengold and B. Munson, *Anal. Chem.*, **42**, 1811 (1970).

(4) R. L. Foltz, 19th Annual Conference on Mass Spectrometry, Atlanta Ga., May 1971, p 142.

(5) The paper by D. F. Hunt and J. F. Ryan III, *Anal. Chem.*, **44**, 1306 (1972), which appeared at the time this communication was submitted for publication discusses the use of argon–water to obtain simultaneous charge exchange–chemical ionization mass spectra without a mention of any other aspect of the use of mixtures of charge exchange and chemical ionization reactant gases.

(6) R. C. Murphy, M. V. Djuricic, S. P. Markey, and K. Biemann, *Science*, **165**, 695 (1969).

(7) G. P. Arsenault, J. J. Dolhun, and K. Biemann, *Anal. Chem.*, **43**, 1720 (1971).

(8) The water-vapor concentration necessary for either simultaneous charge exchange–chemical ionization mass spectra or chemical ionization mass spectra depends on the ion source pressure, the nature of the charge exchange reactant gas, and the mass discrimination inherent to the instrument being used.

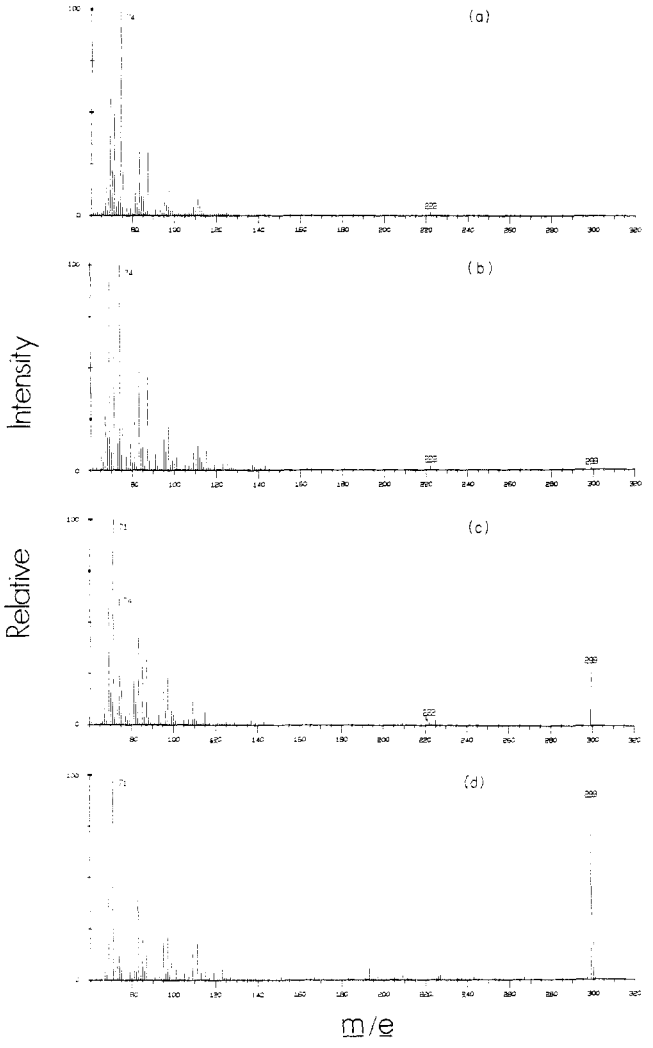

Figure 1. Mass spectra of **1**: (a) electron-impact ionization; (b) helium charge exchange ionization; (c) simultaneous charge exchange (helium)–chemical ionization (water); and (d) chemical ionization (helium/water).

impact ionization which proved so useful in elucidating the structure of **1**.[6]

Helium is first ionized (eq 1) and, in the absence of

$$He + e^- \longrightarrow He\cdot^+ + 2e^- \quad (1)$$

water vapor, the ionization of **1** by charge exchange takes place (eq 2) giving rise to the mass spectrum

$$(1) + He\cdot^- \longrightarrow (1)\cdot^- + He \quad (2)$$

shown in Figure 1b. The concentration of **1** in the high-pressure ion source is not known but is estimated to be too low for any appreciable amount of direct electron-impact ionization of **1** to take place. With helium–water vapor mixtures sufficient water is present for electron-impact ionization of water (eq 3), as well as

$$H_2O + e^- \longrightarrow H_2O\cdot^+ + 2e^- \quad (3)$$

helium (eq 1), to take place. In the high-pressure ion source, water may also be ionized by charge exchange (eq 4) and water ions, whatever their origin (eq 3 and 4),

$$H_2O + He\cdot^+ \longrightarrow H_2O\cdot^+ + He \quad (4)$$

then react with water molecules to give rise to protonated species (eq 5) which are the chemical-ionization reactant ions. Chemical ionization of **1** may then take place as illustrated with H_3O^+ in eq 6. Helium plays

$$H_2O\cdot^+ \xrightarrow{H_2O} H_3O^+ \underset{}{\overset{H_2O}{\rightleftharpoons}} H_5O_2^+ \overset{H_2O}{\rightleftharpoons} H_7O_3^+ \quad (5)$$

$$(1) + H_3O^+ \longrightarrow (1)H^+ + H_2O \quad (6)$$

an important but hidden role in eq 5 and 6 because it makes up the bulk of the gas present at any time in the high-pressure source and serves as a scatter gas. The simultaneous charge exchange–chemical ionization mass spectrum (Figure 1c) was obtained when too little water vapor was present to inhibit charge-exchange ionization of **1** (eq 2), but sufficient water vapor was present for some chemical ionization (eq 6) to take place. The simultaneous charge exchange–chemical ionization mass spectrum of a substance taken with a single high-pressure ion source should bear close resemblance to its simultaneous electron impact–chemical ionization mass spectrum[7] taken with two ion sources provided the electron-impact and charge-exchange mass spectra of the substance are similar. The chemical-ionization mass spectrum of **1** (Figure 1d) was obtained when a sufficient amount of water was present to allow the reactions shown in eq 3–6 to predominate. Alternating between charge exchange (eq 2) and chemical ionization (eq 6) of the sample is possible: rapid changes in the water-vapor concentration of the gas mixture accomplish within a single ion source the equivalent of alternate electron impact–chemical ionization.[7]

Gas mixtures are not limited to helium–water vapor but must consist of a charge-exchange reactant gas as well as a chemical ionization reactant gas. For example, air samples, made up of charge-exchange reactant gases (N_2, O_2, and Ar) and a chemical ionization reactant gas (traces of water vapor), are suitable for study in a high-pressure ion source.[9]

The advantages of mixed charge exchange–chemical ionization reactant gases are far from limited to the production of simultaneous or alternate charge exchange–chemical ionization mass spectra with a single ion source. Chemical-ionization mass spectra may now be obtained with mixed reactant gases using chemical ionization reactant gases which would otherwise rarely be used on account of cost (CD_4) or toxicity (H_2S). For the combination gas chromatograph–mass spectrometer, mixed gases are also ideal because the charge-exchange reactant gas may be used as the carrier gas for gas chromatography and mixed at the outlet of the chromatographic column with the chemical ionization reactant gas. Such an approach is being used in this laboratory and permits the use of any number of suitable gas chromatographic detectors while greatly increasing the number of possible chemical ionization reactant gases for gas chromatography–chemical ionization mass spectrometry[3,10] since compatibility with gas chromatography is no longer required of the chemical-ionization reactant gas. Finally, the use of helium is ideal to further the development of alternate or simultaneous electron impact–chemical ionization mass spectrometry[7] because its low ionization cross section and the ease with which it may be pumped away make

(9) G. P. Arsenault and J. J. Dolhun, 20th Annual Conference on Mass Spectrometry, Dallas, Texas, June 1972, p 253.

(10) G. P. Arsenault, J. J. Dolhun, and K. Biemann, *Chem. Commun.*, 1542 (1970).

it a better choice of bulk gas than others to be present for electron-impact ionization of organic compounds.

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Studies on the Synthesis of Proinsulin. III. Synthesis of Polypeptides Related to the Connecting Peptide Segment of Bovine Proinsulin[1,2]

Sir:

The species variations in the primary structure of porcine,[3,4] bovine,[5-7] and human[8,9] proinsulin are located mostly in the connecting peptide portions and give rise to their unique immunological determinants.

We have previously prepared porcine proinsulin connecting peptide derivatives[10] which were as immunologically active as porcine proinsulin or the connecting peptide of natural origin when measured on an equimolar basis.[1,11] In addition, a rather small peptide fragment located in the central region of the connecting peptide was found to embody the full immunological activity of the complete connecting peptide.[1,11]

This communication describes the syntheses and immunological properties of [59-formyllysine]-bovine proinsulin$_{31-60}$ (I), possessing the entire sequence of the bovine connecting peptide portion, and [50-glycine, 52-alanine,59-formyllysine]-bovine proinsulin$_{31-60}$ (II), possessing the sequence based on an early publication.[5] Differences between I and II are substitutions of amino acids only at positions 50 and 52. Immunological activities of I and II were determined by two different methods with essentially the same results (Chart I).

Using the ethanol precipitation immunoassay[12] with [^{125}I]proinsulin as tracer and a guinea pig antiserum to bovine proinsulin, I cross-reacted with natural bovine proinsulin on an equimolar basis, while II reacted rather poorly (Figure 1). Similar results also were obtained using the double antibody method[13] with ^{125}I synthetic tyrosinated bovine connecting peptide[14] as tracer and a different guinea pig antiserum against bovine proinsulin. In this system, I cross-reacted

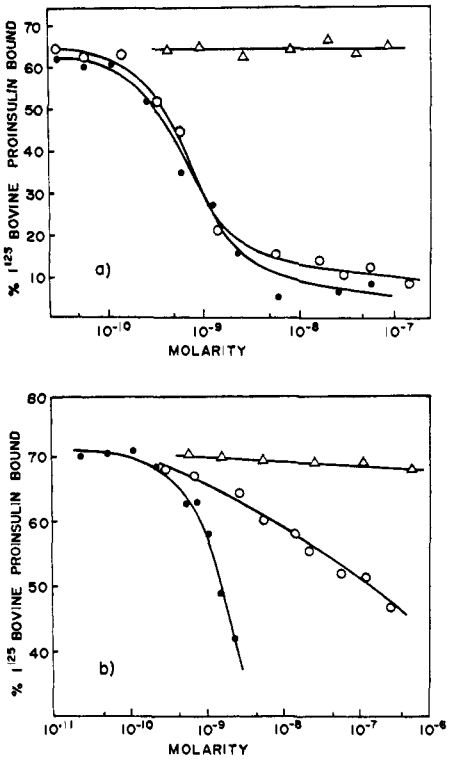

Figure 1. Ethanol precipitation immunoassays: (a) (●) bovine proinsulin, (○) synthetic peptide I, and (△) synthetic peptide III; (b) (●) bovine proinsulin, (○) synthetic peptide II, and (△) synthetic peptide IV.

Chart I

H-Arg-Arg-Glu-Val-Glu-Gly-Pro-Gln-Val-Gly-Ala-Leu-Glu-
31
Leu-Ala-Gly-Gly-Pro-Gly-Ala-Gly-Gly-Leu-Glu-
Gly-Pro-Pro-Gln-Lys(F)-Arg-OH
60

I

H-Arg-Arg-Glu-Val-Glu-Gly-Pro-Gln-Val-Gly-Ala-Leu-Glu-
31
Leu-Ala-Gly-Gly-Pro-Gly-*Gly*-Gly-*Ala*-Leu-Glu-
50 52
Gly-Pro-Pro-Gln-Lys(F)-Arg-OH
60

II

H-Gly-Pro-Gly-Ala-Gly-Gly-Leu-Glu-Gly-Pro-Pro-Gln-
47
Lys(F)-Arg-OH
60

III

H-Gly-Pro-Gly-*Gly*-Gly-*Ala*-Leu-Glu-Gly-Pro-Pro-Gln-
47 50 52
Lys(F)-Arg-OH
60

IV

(1) For part II see N. Yanaihara, T. Hashimoto, C. Yanaihara, M. Sakagami, and N. Sakura, *Diabetes*, **21** (Suppl. 2), 476 (1972).

(2) The amino acids except glycine are of the L configuration: DMSO, dimethyl sulfoxide; DMF, dimethylformamide; Z, benzyloxycarbonyl; OBu^t, *tert*-butyl ester; Boc, *tert*-butoxycarbonyl; F, formyl. R_f^1 and R_f^{II} values refer to the solvent systems: 1-BuOH–AcOH–H_2O (4:1:5) (upper layer) and 1-BuOH–pyridine–AcOH–H_2O (30:20:6:24), respectively. Samples for acid hydrolyses were dried *in vacuo* over P_2O_5 at room temperature, and acid hydrolyses were performed in constant boiling HCl at 110° for 48 hr in sealed tubes. Peptide contents are expressed by average recoveries of amino acids in acid hydrolysates based on formula weight.

(3) R. E. Chance, R. M. Ellis, and W. W. Bromer, *Science*, **161**, 165 (1968).

(4) R. E. Chance, *Diabetes, Proc. Congr. Int. Diabetes Fed., 7th, 1970*, No. 231, 292 (1971).

(5) D. F. Steiner, J. L. Clark, C. Nolan, A. H. Rubenstein, E. Margoliash, B. Aten, and P. E. Oyer, *Recent Progr. Horm. Res.*, **25**, 207 (1969).

(6) D. F. Steiner, S. Cho, P. E. Oyer, S. Terris, J. D. Peterson, and A. H. Rubenstein, *J. Biol. Chem.*, **246**, 1365 (1971).

(7) A. Salokangas, D. G. Smyth, J. Markussen, and F. Sundby, *Eur. J. Biochem.*, **20**, 183 (1971).

(8) P. E. Oyer, S. Cho, J. D. Peterson, and D. F. Steiner, *J. Biol. Chem.*, **246**, 1375 (1971).

(9) A. S. C. Ko, D. G. Smyth, J. Markussen, and F. Sundby, *Eur. J. Biochem.*, **20**, 190 (1971).

(10) N. Yanaihara, T. Hashimoto, C. Yanaihara, and N. Sakura, *Chem. Pharm. Bull.*, **18**, 417 (1970).

(11) R. E. Chance, *Diabetes*, **21** (Suppl. 2), 461 (1972).

(12) L. G. Heding, *Diabetologia*, **1**, 76 (1965).

(13) A. H. Rubenstein, D. F. Steiner, S. Cho, A. M. Lawrence, and L. Kirsteins, *Diabetes*, **18**, 598 (1969).

(14) N. Yanaihara and C. Yanaihara, unpublished data.