Field Desorption Mass Spectrometry Using Needleless Emitters. A Study of Five Synthetic Tripeptides as Hormone Models

Willi Frick,[1a,b] Elisabeth Barofsky,[1a] G. Doyle Daves, Jr.,*[1a]
Douglas F. Barofsky,[1a] Ding Chang,[1b] and Karl Folkers[1b]

Contribution from the Department of Chemistry and Biochemical Sciences,
Oregon Graduate Center, Beaverton, Oregon 97005, and the
Institute for Biomedical Research, The University of Texas at Austin,
Austin, Texas 78712. Received November 2, 1977

Abstract: Five tripeptides (pGlu-His-AspOH, pGlu-His-AsnOH, pGlu-His-AspNH$_2$, pGlu-His-AsnNH$_2$, and pGlu-Asn-HisNH$_2$), models of known and unknown hypophysiotropic hormones, have been synthesized and extensively studied by field desorption mass spectrometry (FDMS) using roughened, needleless tungsten wire emitters. Using needleless emitters rather than conventional carbon microneedle emitters, spectra were obtained from subpicomole quantities of peptide in which ionization occurred by cation attachment (cationization) with the majority of ion intensity borne by MH$^+$, MNa$^+$, and MK$^+$ ions. Low intensity ions of three general types, i.e., dications MH$_2$(Na$_2$)$^{2+}$, ions derived by loss of small molecules [MH(Na) − H$_2$O(NH$_3$,CO$_2$,CONH)]$^+$, and backbone cleavage ions, are observed. This technique is suitable for peptide molecular weight determination with consumption of picogram quantities of material; derivation of other structural information is not readily achieved.

In the decade since Beckey first described the technique,[2a] research in field desorption mass spectrometry (FDMS) has been largely directed toward demonstrating the diversity of chemical structures which are amenable to analysis. Field desorption mass spectra of representative compounds of essentially all major classes of nonpolymeric, polar biomolecules have been reported.[2b,3] While certain generalizations have emerged, the dynamics of field desorption of polar, relatively nonvolatile compounds remain poorly understood.

The analytical aspect of a collaborative research program which includes efforts toward the isolation, structure elucidation, and synthesis of new hypophysiotropic hormones[4-7] has been particularly motivated by the availability[4] of ca. 100 ng of a preparation of a prolactin inhibiting factor from 80 000 hypothalami. In this report, we describe results of analysis by FDMS of five tripeptides. These tripeptides have sequences of pyroglutamic acid, histidine, and aspartic acid and may be considered as "models" of known and of chemically unknown hormones which are being sought through fractionation and hormonal assays. Four of these five tripeptides have a pGlu-His-N-terminal sequence which is also found in the thyrotropin releasing hormone[8] (a tripeptide, pGlu-His-ProNH$_2$) and the luteinizing hormone releasing hormone[9,10] (a decapeptide, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-GlyNH$_2$).

The use of needleless tungsten wire emitters in this investigation (rather than the conventional carbon microneedle emitters[11,12]) has contributed to the understanding of the desorption and ionization processes operative in FDMS of polar molecules.

Experimental Section

Field desorption mass spectra were obtained with a Hitachi-Perkin Elmer RMU-7 single focusing mass spectrometer modified with a field ionization/field desorption ion source and an SSR Model 1120 ion counting detection system. Roughened tungsten wire emitters (8 μm), prepared by sequential oxidation and reduction as described,[12,13] were used. The field desorption source was operated at room temperature with an acceleration potential of 3.6 (3.3) kV and a cathode potential of −6.4 (−6.7) kV. The emitter current was increased linearly at the rate of 6.4 mA/min to a maximum of 20 mA. The five tripeptides were dissolved in 1:1 methanol-water to a concentration of 10^{-4} M. One drop (1-2 nL) of solution, containing ~50 pg of peptide, was placed on the emitter wire with a special microsyringe, which consists of a glass apparatus with a cold finger and an enclosed nichrome wire resistance heater with the enclosed air volume open only through the

fine (i.d. at the tip ~0.025 mm) quartz capillary microsyringe tip. Cooling of the trapped air via submersion of the cold finger in liquid nitrogen while the syringe tip is submerged in a sample solution serves to load the syringe. Similarly, heating the trapped air serves to expel the sample solution as 1-2-nL droplets which are positioned on the emitter by use of a microscope and a micromanipulator.

Spectra were recorded at a scan rate of 37 s/decade beginning when the total ion emission monitor indicated the onset of desorption. For sequential recording the peptide was repeatedly applied to and desorbed from the emitter; each time a different mass interval was selected for recording.

Peptide Synthesis and Characterization. L isomers of the amino acid derivatives were purchased from Beckman, Inc., Palo Alto, Calif., and Peninsula Laboratories, San Carlos, Calif. Amino acid analyses were carried out on a Beckman amino acid analyzer, Model 119, after hydrolysis of the samples in 6 M HCl overnight in evacuated sealed tubes at 130 °C. Syntheses were done by the Merrifield solid phase procedure with a Beckman Model 990 automatic Synthesizer. α-Amino groups were protected by the *tert*-butyloxycarbonyl group (Boc). The side-chain protecting groups were benzyl (Bzl) for Asp, *p*-toluenesulfonyl (Tos) for His, and benzyloxycarbonyl (Z) for pGlu. Following deprotection and cleavage from the resin, the crude lyophilized peptide was subjected to gel filtration on a 2 × 100 cm column of Sephadex G-10 eluted with 1.3% AcOH in H$_2$O or with *n*-BuOH–AcOH–H$_2$O (4:1:5) and detection of the peptide by TLC. When necessary further purification using a 1.5 × 80 cm column of LH-20 elution with the system *n*-BuOH in H$_2$O (6:100) was carried out. Homogeneity was assured by thin-layer chromatography. The peptides synthesized and their chromatographic properties are included in Table I.

Results

Recording of Spectra. Typical field desorption mass spectra of the peptides studied are shown in Figures 1 and 2. Due to the small amount of sample on the emitter, a sufficiently intense ion current for recording can be maintained only for a short period of time. Synchronization of the recording with the desorption profile allows the recording of spectra at a particular stage of sample desorption (see Figures 1a and 1b).

Desorption of an entire sample, once initiated at a specific emitter temperature, usually occurs in a time interval shorter than that required to scan the entire mass range. For extended mass ranges, it was found necessary to record selected regions in sequential experiments in order to obtain sufficient intensities of low abundance ions for accurate mass assignment (see Figure 2). Due to these experimental circumstances, inter-
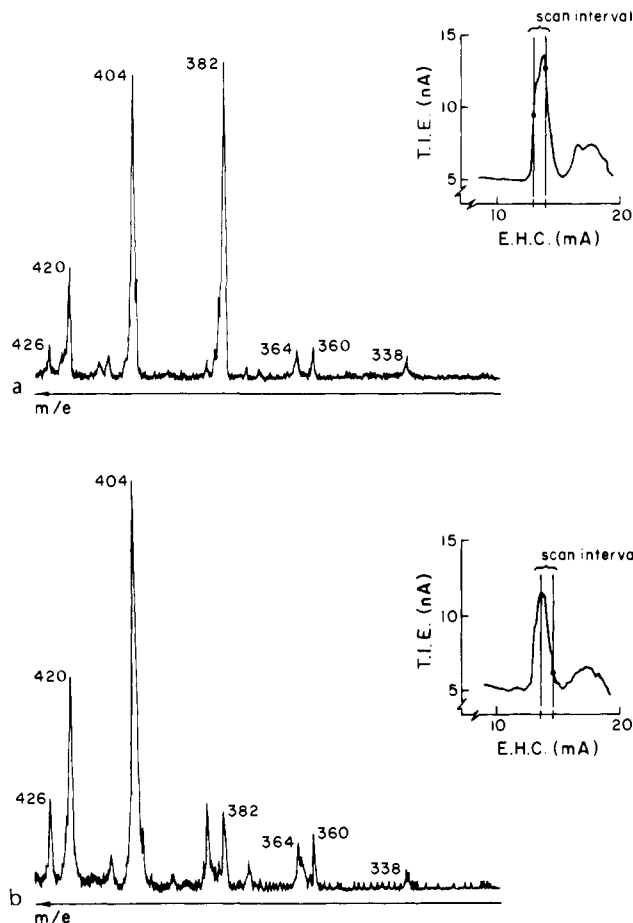
Figure 1. Relationship between sample desorption profiles (plots of total ion emission. T.I.E., vs. emitter heating current, E.H.C., see insets) and field desorption mass spectra of pGlu-His-AspOH in the pseudomolecular ion (cationized molecular ion) region: (a, top) early stage of sample desorption; (b, bottom) late stage of sample desorption (compare sample desorption profiles). Ion assignments are in Table II.

Table I. Thin-Layer Chromatographic Properties of Model Tripeptides[a]

| peptide | R_f values in solvent systems | | |
	1	2	3
pGlu-His-AspOH	0.33	0.41	0.34
pGlu-His-AsnOH	0.27	0.34	0.25
pGlu-His-AspNH₂	0.28	0.35	0.26
pGlu-His-AsnNH₂	0.28	0.66	0.40
pGlu-Asn-HisNH₂	0.29	0.70	0.45

[a] Silica gel G plates were used. Solvent systems were (v/v): (1) n-BuOH-AcOH-H_2O (1:1:1); (2) EtOAc-pyridine-AcOH-H_2O) (5:5:1:3); (3) n-BuOH-AcOH-H_2O-pyridine (15:3:12:10). Spots were visualized using Pauly, I_2-chlorine, and o-toluidine reagents. For each purified peptide, single spots were observed in each system with each detection reagent.

pretation of peak heights in terms of ion abundances can be made only to a limited degree over narrow mass-range intervals ($\sim\pm25$ amu).

Peptide Desorption Profiles. Monitoring of total ion emission as the emitter heating current increases in accord with a preselected program produces a desorption profile (for examples, see Figure 1). The profiles for the five tripeptides under investigation are essentially indistinguishable; changes in

functional groups (COOH, $CONH_2$) or in amino acid sequence (pGlu-His-AsnNH₂ and pGlu-Asn-HisNH₂) produce no observable effect on desorption from the emitter. Using a new emitter, maximum peptide desorption occurs at an emitter heating current of 10-10.5 mA. During a typical emitter lifespan of 50-60 analyses, the position of the peptide desorption maximum gradually shifts to a heating current of ~8.5 mA.

Ion Identification. The principal ions observed in spectra of the five tripeptides are recorded in Table II. It is particularly noteworthy that these spectra appear to consist almost entirely of even electron ions. In no instance was a molecular ion ($M^+\cdot$) observed; instead, spectra recorded during early stages of sample desorption (see Figure 1a) exhibit intense pseudomolecular ions (MH^+) and ions (MNa^+ and MK^+) formed by cationization of the peptide by alkali metal ions,[14] presumably present as contaminants. Spectra recorded in the decreasing phase of the desorption profile (see Figure 1b) exhibit relatively intense ions which correspond to losses of small molecules (H_2O, NH_3, CO_2, or CONH) from the peptide with subsequent cationization of the resulting anhydro or descarboxy peptide. The multiplicity of ions which result from this general process is evident from Figure 3. In the spectra of these peptides (Table II) dicationization[15] is observed giving rise to ions, exemplified by MNa_2^{2+}, which are easily identified in the case of peptides of odd molecular weight by virtue of their "half mass" m/e values. Ions are also observed in the spectra of each of the peptides (Table II) which accords with the assignment [$MNa_2 - H$]. For peptides bearing one or more carboxyl groups, e.g., pGlu-His-AspOH or pGlu-His-AsnOH, it is convenient to visualize this ion as a cationized peptide
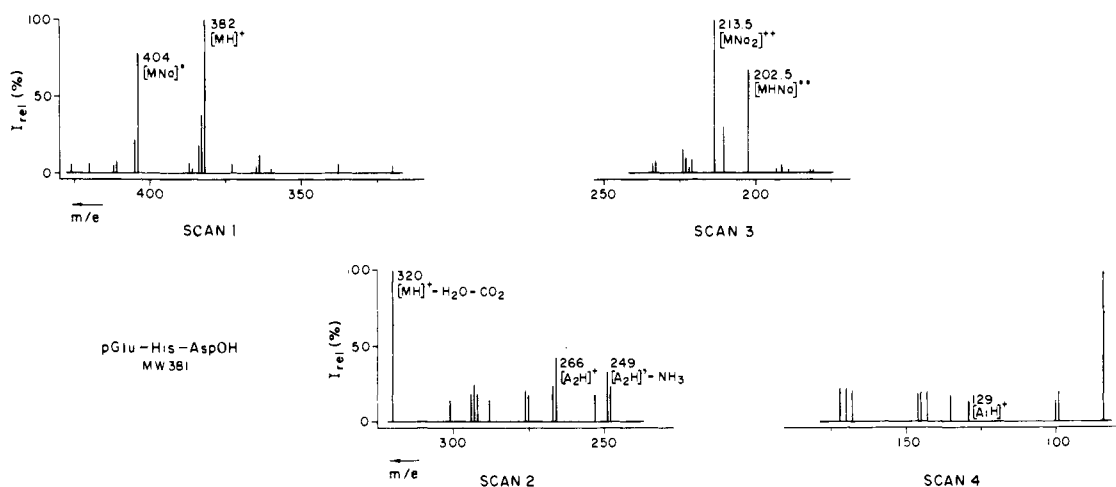

Figure 2. Composite field desorption mass spectrum of pGlu-His-AspOH obtained by scanning different preselected mass ranges during field desorption of four separate samples (see Experimental Section). Ion assignments not included in the figure are recorded in Table II.

Table II. Selected Ions Observed in the FDMS of Model Tripeptides

	pGlu-His-AspOH mol wt 381	pGlu-His-AsnOH mol wt 380	pGlu-His-AspNH₂ mol wt 380	pGlu-His-AsnNH₂ mol wt 379	pGlu-Asn-HisNH₂ mol wt 379
$[2Na + M - H]^+$	426	425	425	424	424
$[K + M]^+$	420	419	419	418	418
$[Na + M]^+$	404	403	403	402	402
$[Na + M - H_2O]^+$ [a]	386	385	385	385	385
$[H + M]^+$	382	381	381	380	380
$[H + M - H_2O]^+$ [a]	364	363, 364	363, 364	363	363
$[Na + M - CO_2]^+$ [b]	360	359, 360	359, 360	359	359
$[H + M - 2H_2O]^+$ [a]	346	345, 346, 347	345, 346, 347		345
$[H + M - CO_2]^+$ [b]	338	337, 338	337, 338	337	337
$[H + M - CO_2, H_2O]^+$ [a,b]	320	320	319, 320	319	319
$[Na + A_2]^+$ [c]	288	288	288	288	265
$[H + A_2]^+$ [c]	266	266	266	266	243
$[H + Z_1]^+$ [c]		253	253	252	252
$[H + A_2 - H_2O]^+$ [a,c]	248, 249	248	248, 249	248, 249	225, 226
$[2Na + M]^{2+}$	213.5	213	213	212.5	212.5
$[H + K + M]^{2+}$	210.5	210		209.5	
$[H + Na + M]^{2+}$	202.5	202	202	201.5	201.5
$[2H + M]^{2+}$	191.5	191	191		190.5
$[Na + A_1]^+$ [c]	151			151	
$[H + A_1]^+$ [c]	129	129	129	129	129

[a] Loss of NH_3 instead of H_2O occurs from C-terminal amides. [b] Loss of CONH instead of CO_2 occurs from C-terminal amides. [c] See Figure 3 for notation of ion structure.

sodium carboxylate, an ion type well known[14] in field desorption mass spectrometry. However, ions of composition $[MNa_2 - H]^+$ appear in the spectra of the peptide amides pGlu-His-AsnNH₂ and pGlu-Asn-HisNH₂ which possess no carboxyl groups; presumably, in these cases it is the acidic histidine N–H which is replaced by sodium.

In addition to those ions resulting from peptide cationization, with or without concomitant loss of one or more small molecules (H_2O, NH_3, CO_2, or CONH), ions of low relative abundance are observed which result from disruption of the peptide backbone. In spectra of pGlu-His-AspOH (Figures 2 and 3), ions at m/e 129 and (occasionally) 151 are observed which are indicative of the pyroglutamyl N terminus of the peptide. The C-terminal desamino dipeptide, formed by the elimination reaction, gives rise to an ion at m/e 276. A similar elimination forming pGlu-HisNH₂ is evidenced by ions in the spectrum occurring at m/e 288, 266, and 248. In this case, no ion was observed which is assignable to the corresponding C-terminal fragment.

Comparison of Spectra of Peptide Isomer Pairs. Included in this study (Table II) are two isomer pairs. One pair, pGlu-His-AsnOH and pGlu-His AspNH₂, differs only in the functionalization of the C-terminal (AsnOH, AspNH₂) residue. These peptides are indistinguishable by either their desorption behavior or their field desorption mass spectra. In the spectra of each of these compounds, ions are observed which correspond to expulsion of both H_2O and NH_3 and both CO_2 and CONH, suggesting that there is little or no position specificity for these elimination reactions.

The second pair, pGlu-His-AsnNH₂ and pGlu-Asn-HisNH₂, consists of sequence isomers, and their spectra are distinguishable and indicative of the differing sequences. In each spectrum, an ion is observed at m/e 129 indicative of the common pyroglutamyl N terminus (cf. Figure 3). A corresponding C-terminus ion at m/e 252 is also present in each spectrum. The spectrum of each of the sequence isomers exhibits a unique ion series, m/e 288, 266, and 248 for pGlu-His-AsnNH₂ and m/e 265, 243, and 226 for pGlu-Asn-HisNH₂. These distinguishing ion series arise by amide elimination reactions at the His-Asn and Asn-His linkages, respectively, and not only differentiate the isomers, but with the identification of the N terminus as pGlu (ion at m/e 129), define the amino acid sequences of the two peptides. These
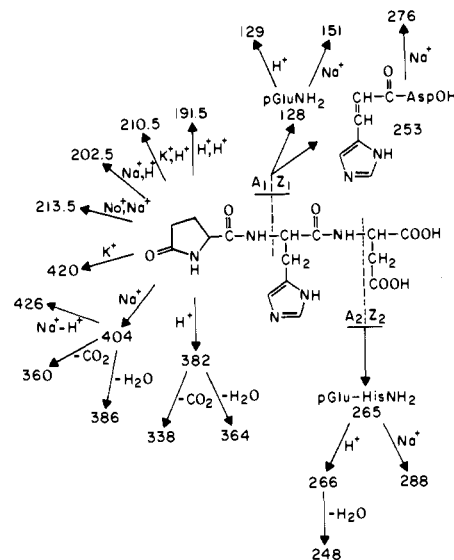

Figure 3. Structure assignments of selected ions occurring in the field desorption mass spectrum of pGlu-His-AspOH.

amino acid sequence determining ionizations are shown graphically in Figure 4.

Discussion

The first published field desorption mass spectrum of a peptide was that of glycylglycine reported by Beckey et al. in 1971.[16] Winkler and Beckey[17] subsequently reported several additional peptide spectra including that of a nonapeptide. Asante-Poku[18] and Asante-Poku et al.[19] have also reported a number of peptide spectra including, as well, one of a nonapeptide (bradykinin). Frick et al.[5] reported the effective use of peptide methylation–methanolysis and FDMS analysis to derive sequence information. Dell et al.[20] used FDMS to determine the molecular weight of a peptide antibiotic, echinomycin. Winkler et al.,[21] in an FDMS analysis of glucagon, a peptide of 29 amino acid residues, recorded ions with m/e values >4000. Rinehart et al.[22] have obtained cationized molecular ions for a series of peptide antibiotics of molecular weights 1500–2000.
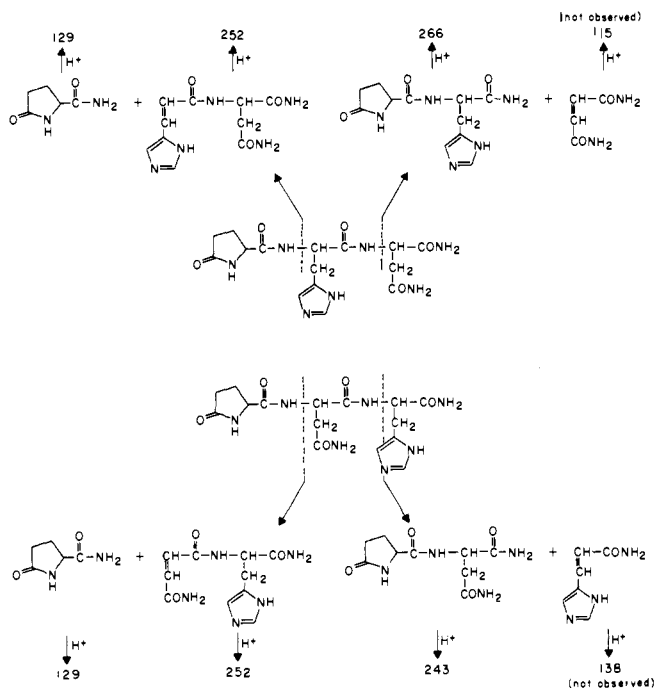
Figure 4. Ions present in the field desorption mass spectra of the isomeric peptides pGlu-His-AsnNH$_2$ and pGlu-Asn-HisNH$_2$ which distinguish the isomers and allow unique amino acid sequences for each to be assigned.

The present study was undertaken to assess several aspects of FDMS for use in total structure analysis of very small (nanogram) quantities of biologically derived oligopeptide fractions obtained in connection with the search for new hypophysiotropic hormones.[4-7] We were able to utilize sample solutions of 10 μL (or less) total volume and consume only picogram quantities of sample to obtain full field desorption mass spectra of the model tripeptides by sequentially recording preselected spectrometric regions. The spectra obtained are complex (Table II, Figures 1–3); however, the molecular weights of the model peptides could be deduced since the most abundant ions were usually MH$^+$ and MNa$^+$ and other ions of lower abundance indicative of peptide molecular weight could often be identified. All spectra we obtained exhibited ions formed by sodium and potassium ion attachment although these alkali metal ions were not deliberately added. Ions other than those indicative of compound molecular weight are of two types. Those derived by loss of small molecules are of little structural significance. Those derived by cleavage of the peptide backbone[23] do contain significant structural information but, under the conditions of our study, were of very low abundance and, owing to the overall complexity of the spectra, probably could not be identified without important independent knowledge of the peptide structure (e.g., the amino acid composition). We conclude that FDMS using needleless emitters possesses the high sensitivity required for molecular weight determination of submicrogram quantities of biologically isolated oligopeptides but that further structural information is not readily available by this technique. We suggest that it is best used in conjunction with other highly sensitive techniques such as those we have developed for amino acid analysis,[6] FDMS analysis of oligopeptide methylation-methanolysis product mixtures,[5] and electron ionization mass spectrometric analysis of underivatized peptides.[24,25]

It is apparent that caution is necessary in comparing results obtained in different studies since FDMS can be affected critically by such factors as the nature of the emitter used,[12,26,27] the amount of sample adsorbed onto the emitter, the solvent from which it is adsorbed,[28] the nature and extent

of impurities in the sample or solvent,[28-30] the presence or absence of particular cations,[27,31,32] the rate and extent of emitter heating,[33-35] and the method used to record spectra.[36,37]

In considering the results of our present study, it is particularly important to take note of the fact that oxidatively roughened (needleless) tungsten wire emitters[12,13] were used, rather than the more typical benzonitrile activated carbon microneedle emitters used in previous studies of peptide FDMS.[5,14,16-21,23] Use of needleless emitters enhances ion formation by cation attachment (cationization) at the expense of other modes of ionization.[27] Essentially all ions observed in our peptide spectra are, in a formal sense, derived by cationization of a neutral species. Implications of this fact are that (a) all ions are formed on the emitter surface, (b) they possess insufficient internal energy to permit fragmentation reactions,[27,38-40] and, therefore, (c) they directly reflect thermochemical events occurring on the emitter surface.

The first evidence that FDMS involves ionization modes, which do not require prior sample migration to high-field regions at the microneedle tips, was provided by Barofsky and Barofsky,[12] who observed that ionization occurs in "bursts" which correlate with emitter temperature increases. Röllgen and Schulten[27] postulated that alkali ion attachment (cationization) "results from a surface reaction in the transition region between thermal and field ionization." Holland et al.[41] noted that sample desorption from carbon microneedle emitters appeared to be field independent and postulated ion formation via "chemical attachment reactions in a thin fluidized system" on the emitter surface.

References and Notes

(1) (a) Department of Chemistry and Biochemical Sciences, Oregon Graduate Center; (b) Institute for Biomedical Research, The University of Texas at Austin.
(2) (a) H. D. Beckey, *Int. J. Mass Spectrom. Ion Phys.*, **2**, 500 (1969); (b) H. D. Beckey and H. R. Schulten, *Angew. Chem., Int. Ed. Engl.*, **14**, 403 (1975).
(3) H. D. Beckey and H. R. Schulten, *Z. Anal. Chem.*, **273**, 345 (1975).
(4) T. Greibrokk, J. Hansen, R. Knudsen, Y. K. Lam, and K. Folkers, *Biochem. Biophys. Res. Commun.*, **67**, 338 (1975).
(5) W. Frick, G. D. Daves, Jr., D. F. Barofsky, E. Barofsky, G. H. Fisher, D. Chang, and K. Folkers, *Biomed. Mass Spectrom.*, **4**, 152 (1977).
(6) W. Frick, D. Chang, K. Folkers, and G. D. Daves, Jr., *Anal. Chem.*, **49**, 1241 (1977).
(7) Y.-K. Lam, R. Knudsen, K. Folkers, W. Frick, G. D. Daves, Jr., D. F. Barofsky, and C. Y. Bowers, *Biochem. Biophys. Res. Commun.*, **81**, 680 (1978).
(8) K. Folkers, F. Enzmann, J. Boler, C. Y. Bowers, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **37**, 123 (1969).
(9) H. Matsuo, Y. Baba, R. M. G. Nair, A. Arimura, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **43**, 1334 (1971).
(10) H. Sievertsson, J. K. Chang, C. Bogentoft, B. Currie, K. Folkers, and C. Y. Bowers, *Biochem. Biophys. Res. Commun.*, **44**, 1566 (1971).
(11) H. D. Beckey, E. Hilt, and H. R. Schulten, *J. Phys. E*, **6**, 1043 (1973).
(12) D. F. Barofsky and E. Barofsky, *Int. J. Mass Spectrom. Ion Phys.*, **14**, 3 (1974).
(13) The actual sequence used to prepare emitters is: (1) the emitter is heated in a hydrogen atmosphere to clean the surface, (2) then it is heated in a hydrogen–water vapor mixture to oxidize the surface, and (3) it is reduced by heating in a hydrogen atmosphere.[12]
(14) H. R. Schulten and F. W. Röllgen, *Org. Mass Spectrom.*, **10**, 649 (1975).
(15) H. R. Schulten and H. M. Schiebel, *Z. Anal. Chem.*, **280**, 139 (1976).
(16) H. D. Beckey, G. Hoffmann, K. H. Maurer, and H. U. Winkler, *Adv. Mass Spectrom.*, **5**, 626 (1971).
(17) H. U. Winkler and H. D. Beckey, *Biochem. Biophys. Res. Commun.*, **46**, 391 (1972).
(18) S. Asante-Poku, Ph.D. Thesis, University of Windsor, Windsor, Ontario, Canada, 1975.
(19) S. Asante-Poku, G. W. Wood, and D. E. Schmidt, Jr., *Biomed. Mass Spectrom.*, **2**, 121 (1975).
(20) A. Dell, D. H. Williams, H. R. Morris, G. A. Smith, J. Feeney, and G. C. K. Roberts, *J. Am. Chem. Soc.*, **97**, 2497 (1975).
(21) H. U. Winkler, R. H. Beuhler, and L. Friedman, *Biomed. Mass Spectrom.*, **3**, 201 (1976).
(22) K. C. Rinehart, Jr., J. C. Cook, Jr., H. Meng, K. L. Olson, and R. C. Pandey,

Nature (London), **269**, 832 (1977).

(23) Biemann (K. Biemann, "Mass Spectrometry, Organic Chemical Applications", McGraw-Hill, New York, N.Y., 1962, pp 294–296) observed identical peptide backbone cleavage reactions during early studies using electron ionization mass spectrometry and high temperatures for sample volatization.

(24) W. R. Anderson, Jr., W. Frick, G. D. Daves, Jr., D. F. Barofsky, I. Yamaguchi, D. Chang, K. Folkers, and S. Rosell, *Biochem. Biophys. Res. Commun.*, **78**, 372 (1977).

(25) W. R. Anderson, Jr., W. Frick, and G. D. Daves, Jr., *J. Am. Chem. Soc.*, **100**, 1974 (1978).

(26) M. D. Migahed and H. D. Beckey, *Int. J. Mass Spectrom. Ion Phys.*, **7**, 1 (1971).

(27) F. W. Röllgen and H. R. Schulten, *Z. Naturforsch.*, **30**, 1685 (1975).

(28) H. U. Winkler and H. D. Beckey, *Org. Mass Spectrom.*, **6**, 655 (1972).

(29) M. Anbar and G. A. St. John, *Anal. Chem.*, **48**, 198 (1976).

(30) G. W. Wood, P.-Y. Lau, and N. Mak, *Biomed. Mass Spectrom.*, 1, 425

(1974).

(31) H. J. Veith, *Angew. Chem., Int. Ed. Engl.*, **88**, 762 (1976).

(32) F. W. Röllgen and H. R. Schulten, *Org. Mass Spectrom.*, **10**, 660 (1975).

(33) H. Knöppel, *Int. J. Mass Spectrom. Ion Phys.*, **4**, 97 (1970).

(34) D. Kümmler and H. R. Schulten, *Org. Mass Spectrom.*, **10**, 813 (1975).

(35) H. U. Winkler, W. Neumann, and H. D. Beckey, *Int. J. Mass Spectrom. Ion Phys.*, **21**, 57 (1976).

(36) H. R. Schulten and H. D. Beckey, *Org. Mass Spectrom.*, **7**, 867 (1973).

(37) H. Budzikiewicz and M. Linscheid, *Biomed. Mass Spectrom.*, **4**, 103 (1977).

(38) R. C. Dougherty, *J. Am. Chem. Soc.*, **90**, 5780 (1968).

(39) E. K. Fields and S. Meyerson, *J. Am. Chem. Soc.*, **88**, 2836 (1966).

(40) J. T. Bursey, M. M. Bursey, and D. G. I. Kingston, *Chem. Rev.*, **73**, 191 (1973).

(41) J. F. Holland, B. Soltmann, and C. C. Sweeley, *Biomed. Mass Spectrom.*, **3**, 340 (1976).

An Unconventional Approach to Structure Determination of Isomeric Cage Compounds

Benzion Fuchs* and Mordechai Pasternak

Contribution from the Department of Chemistry, Tel-Aviv University, Ramat-Aviv, Tel-Aviv, Israel. Received March 15, 1978

Abstract: After the failure of conventional chemical and physical attempts for structural differentiation between C_{2v} and D_{2h} symmetries in the bishomocubane dione cage compounds of photochemical origin (**4** and **5**), this was achieved using an approach which combines mass spectrometry with isotopic labeling techniques and symmetry considerations. The isomeric cages of C_2 symmetry (**3**) exhibit also interesting characteristic fragmentation patterns, under electron impact, which are inter alia of diagnostic value.

We are, for some time now, engaged in a study of a versatile and interesting class of compounds, namely, cyclopentadienones (**1**) and their dimers (**2**).[1-4] In the course of an investigation of the photochemical behavior of the substituted
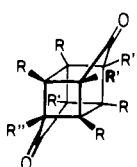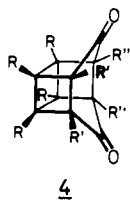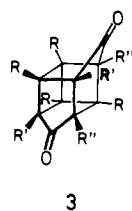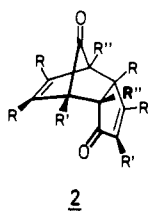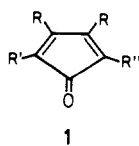
dimer (**2b**)[2] we were faced with a structural problem seemingly simple to deal with, based on our experience with the unsubstituted dimer (**2a**).[4]

Irradiation of **2b** provides two photoproducts, in temperature-, phase-, and wavelength-dependent yields.[2] Both are isomeric with the starting material and we list in Table I the spectroscopic properties that one could use for structure determination. Thus, UV and IR spectroscopic data evidently point to the absence of conjugated phenyl groups and to the presence of only saturated, strained carbonyls. This indicates that we deal with cage compounds of three possible structures, **3b**, **4b**, and **5b**. The one having structure **3b** was given away by its NMR spectrum in which two methyl-proton resonances occur, one for each equivalent pair of methyls in **3b** as expected for a dissymmetric structure (point symmetry C_2). The second photoproduct exhibits only one singlet for all 12 methyl protons indicating a symmetric structure but not discriminating between **4b** of point symmetry C_{2v} and the centrosymmetric form **5b**, of symmetry D_{2h}.

Unfortunately, the symmetric product is a high-melting compound of extremely low solubility in all organic solvents and exceedingly inert to chemical agents, although thermally unstable above ca. 100 °C when it reverts to **2b**.[2] This behavior is understandably due to its high molecular weight and symmetry as well as steric crowding around potential chemical reaction centers. Thus, it withstood all nucleophilic attack as attempted with various reagents and the only reaction that could be carried out was reduction by $LiAlH_4$ in boiling tetrahydrofuran to give a diol, which also resisted derivatization and hence structural assignment.[2] Moreover, even an x-ray diffraction analysis could not be attempted because no proper crystals could be grown owing to the low solubility of both these compounds.

*	a	b	c	e	f
R	H	Ph	Ph	Ph-d_5	Ph-d_5
R'	H	Me	Me	Me-d_3	Me-d_3
R''	H	Me	H	Me-d_3	H

*Notation used throughout the paper (in text and formulas)