possibility that the line is due to an impurity in WO_3, but then it should have appeared also in the more dilute bronzes examined, $H_{0.03}WO_3$ and $H_{0.05}WO_3$. The line is probably not due to W^{5+}, which generally shows g factors in the range 1.4–1.7.

Since cavity loading was not a problem with $H_{0.10}WO_3$, it can be concluded that this composition is not highly conducting. Arguments have previously been given[8] for believing that the metallic bronzes should go over to semiconducting behavior when x in M_xWO_3 drops below approximately 0.25. The critical composition depends on the effective mass of the electron and may be smaller for H_xWO_3 than for M_xWO_3. In any case, there would be good reason for believing that electrons in dilute H_xWO_3 are localized enough to give esr absorption and semiconductivity. The closeness of the g value to 2 suggests localization at the H atoms. A detailed nmr study might be informative, but line broadening makes the problem a difficult one.

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Negative Ion Mass Spectra of Benzene, Naphthalene, and Anthracene. A New Technique for Obtaining Relatively Intense and Reproducible Negative Ion Mass Spectra

Sir:

Negative ion mass spectra taken at low pressures and high electron energies are notoriously weak and nonreproducible.[1] As a consequence of these factors negative ion phenomena have received very little attention in the mass spectra of organic compounds. We herein report a new general technique for obtaining negative ion mass spectra in conventional mass spectrometers which substantially increases both the reproducibility and intensity of the spectra.

Negative ion mass spectra are the result of three ion-formation processes: (1) resonance capture; (2) dissociative resonance capture; and (3) heterolytic fission (ion-pair formation). The abundance of products from these processes depends on the sample pressure, electron current, and energy.

At high emission voltages (50–100 eV) process 3 depends on the transition probability for excitation and is independent of electron energy. Processes 1 and 2 are critically dependent on the energy of the impacting electron. Electrons in the appropriate energy region for resonance capture processes (0–10 eV) are generated as secondary electrons in the formation of positive ions. The magnitude of the secondary electron current is a function of sample pressure. Thus processes 1 and 2 may show a quadratic dependence on pressure.[1] At low pressures the intensities of resonance capture ions (process 1) often show a cubic dependence because of the need for collisional stabilization of the initial ion.[1] These three independent nonconcurrent modes of formation of negative ions by electron impact account for the nonreproducibility and in part the low intensity of negative ion mass spectra.

By use of high pressures of organic molecules intense negative ion mass spectra may be obtained.[2,3] These spectra are usually complicated by ion–molecule and ion–ion reactions,[3] and high pressures of organic molecules will soon destroy a general use analytical mass spectrometer.

Analytical mass spectrometers, particularly those with differential pumping like the MS-902, can be operated at high pressure of nonreactive gases. It should be possible to enhance the negative ion mass spectrum of a sample with such a gas. If the enhancing gas is at least an order of magnitude more abundant than the compound under investiagtion, three things should result. The abundance of secondary electrons should be increased. At relatively high pressures ($>10^{-5}$ torr) the secondary electron current can become almost as large as the primary current. Furthermore there will be a spectrum of electron energies due to secondary and degraded primary electrons that should cover the entire range of energies from zero to the primary electron energy. The energy spectrum of the secondary and degraded primary electrons should be relatively constant from sample to sample, and ion-forming processes 1, 2, and 3 should all be approximately first order in sample pressure. Processes 1 and 2 should be respectively second and first order in enhancing gas pressure.

Table I presents negative ion mass spectra of benzene, naphthalene, and anthracene which were obtained with an A.E.I. MS-902 under the following conditions: sample pressure,[4] 1×10^{-6} torr; nitrogen pressure,[4] 6×10^{-5} torr (under these conditions the analyzer pressure was $1.0 \pm 0.1 \times 10^{-6}$ torr); electron emission, 70 eV with 485 μA at the electron trap; ion repeller, 0.0 ± 0.05;[5] source housing, -8 kV; electron multiplier, 3000 V. The mass scale was determined by direct comparison with standard spectra[6] obtained for either positive or negative ions.

The second column in Table I presents the enhancement factors for benzene using nitrogen. This tech-

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[1] C. E. Melton in "Mass Spectrometry of Organic Ions," F. W. McLafferty, Ed., Academic Press, New York, N. Y., 1963.

[2] R. Tummler and G. Liebisch, *Chem. Tech.* (Berlin), **19**, 624 (1967).

[3] R. C. Dougherty, submitted for publication.

[4] Source pressure is measured just above the source liquid nitrogen trap on the MS-902. The actual pressure in the source chamber is certainly higher.

[5] The secondary electron energy spectrum critically depends on the repeller potential. If the repeller is adjusted to a modest negative value (~ -40 V) with respect to the source housing, the efficiency of ion draw-out is increased; however, resonance capture processes are diminished because of the acceleration of the secondary electrons.

[6] The appropriate field reversals for negative ion studies were accomplished by using a Power Designs Pacific Model No. HV-1543 power supply for ± 8 kV and a Cohu Model 326 power supply of ± 270 V for the electrostatic analyzer. Using these supplies the low-resolution mass scale for a given scan rate and recorder speed shows virtually no variation over a period of 2 weeks, and we have successfully calibrated negative ion spectra by comparison with corresponding positive ion records with results that are entirely consistent with internal standard techniques.

nique allows one to immediately distinguish the ions that are formed by process 1 or 2 as opposed to 3. High enhancement, as with *m/e* 26, clearly indicates a resonance capture process. Enhancement factors between 1.5 and 2.0 could indicate some resonance capture or collisional stabilization of the negative ion. Enhancement factors are not given for naphthalene or anthracene because these compounds were introduced through the solid probe and the spectra in the absence of an enhancing gas show variable relative intensities. In both cases, however, parent ion clusters were not observed immediately prior to and following the nitrogen-enhanced spectra recorded here, in which parent ions are prominent. In the presence of N_2 the molecule ions increased in relative and absolute intensity in the series $C_6H_6 < C_{10}H_8 < C_{14}H_{10}$, in accord with the increasing electron affinity in the series.[7] Ion–molecule reactions can still occur under these conditions, *e.g.*, *m/e* 79 in the spectrum of benzene.

Table I. Nitrogen-Enhanced Negative Ion Mass Spectra of Benzene, Naphthalene, and Anthracene[a]

	Relative intensity			
m/e	Benzene[b]	Enhancement factors for benzene[c]	Naphthalene[d]	Anthracene[d]
12	1.5 ± 0.3		6.0	11.0
24	10.2 ± 1.9	1.3	7.1	17.0
25	19.3 ± 1.6	1.2	8.5	20.5
26	100.0	49.8	100.0	100.0
27	3.0 ± 0.3	49.5	2.0	2.0
36	2.3 ± 0.2	1.1	2.2	3.6
48	2.9 ± 0.1	1.0	1.0	2.2
49	3.1 ± 0.2	1.0	4.5	6.6
50	3.0 ± 0.2	3.0		7.3
60	0.4 ± 0.1	1.2		0.1
61				0.5
62				0.4
66				0.9
72	1.5 ± 0.2	1.2	0.8	4.7
73	1.0 ± 0.1	1.2	0.1	0.6
74	0.5 ± 0.1	1.0		2.0
79	0.2 ± 0.1	20.1		
84				0.6
96				2.0
97				1.8
121				0.9
127				2.3
128			2.8	2.8
129			0.7	0.7
145				1.4
177				1.0
178				3.5
179				2.4
180				1.0

[a] Intensities below 0.1 neglected because of the high gain factor and concurrent noise. [b] Sample pressure, 1.0 ± 0.05 × 10^{-6} torr; nitrogen pressure, 6.0 ± 0.2 × 10^{-5} torr. Average of ten spectra with average deviations. [c] Benzene, sample pressure, 1.0 ± 0.05 × 10^{-6}; intensity = 1.0, compared with spectra taken under conditions of column 1,[b] which were recorded immediately after the reference spectra. Ratios of absolute intensities are averages of six spectra; deviations were less than 10%. [d] Sample pressure, 1.0 ± 0.2 × 10^{-6} torr; nitrogen pressure, 6.0 ± 0.2 × 10^{-5} torr. Averages of six spectra; average deviations were less than 10% of the relative intensity.

Gas enhancement of negative ion mass spectra appears to offer a substantial improvement in the prospects for use of negative ion mass spectra as models for reduction processes and radiolysis reactions. If enhanced negative ion mass spectra are tabulated for these purposes, it would be useful if a specific gas and pressure were generally adopted.

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Solid-Phase Peptide Coupling

Sir:

Stepwise solid-phase peptide synthesis[1] has provided a rapid method for preparation in high yield of amino acid sequences of several peptide hormones.[2–6] In applying this approach to the total synthesis of the enzyme staphylococcal nuclease,[7] we have prepared protected peptide fragments by treating the *t*-butyloxycarbonyl (BOC) hydroxysuccinimide ester of glutamic acid or of aspartic acid with the peptide cleaved from the Merrifield polymer[8] and, in a special case, by hydrazinolysis of the protected peptide from the polymer as the hydrazide.[9] These fragments may be purified before coupling to give longer sequences.

We report here the efficient coupling of soluble protected di-, tri-, and tetrapeptides to the amino terminus of another peptide attached to the Merrifield polymer. To minimize racemization of the carboxyl component, N-ethyl-5-phenylisoxazolium-3′-sulfonate (NEPIS),[10] N,N′-dicyclohexylcarbodiimide (DCC) plus N-hydroxysuccinimide (HOSu),[11] and the azide coupling methods were applied.

In a typical experiment, 106 mg (0.10 mmol) of *t*-BOC-γ-benzyl-L-glutamyl-ε-carbobenzoxy(Z)-L-lysyl-ε-Z-L-lysyl-O-benzyl-L-serine was activated with NEPIS (25.3 mg, 0.10 mmol) and 0.10 mmol of triethylamine in dimethylformamide, then added in fourfold excess to H_2N-L-leucyl-L-prolyl polymer (0.025 mmol, 83.3 mg) suspended in dimethylformamide. The reaction mixture was shaken at room temperature for 3 hr, and the resulting peptide polymer was washed with dimethylformamide, methanol, and ether to remove the soluble reactant, then was cleaved and deprotected with hydrogen bromide in trifluoroacetic acid at room temperature for 90 min. The polymer was removed by filtration and washed with trifluoroacetic acid and methylene chloride. The hexapeptide HBr · H_2N-Glu-Lys-Lys-Ser-Leu-Pro-OH was obtained from

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