pepsin modified by I (pepsin M-I) or by II (pepsin M-II) were examined at 25.0° from pH 3.05 to 5.8, under conditions of enzyme in excess, following *p*-nitrophenol release at 320 nm with a Cary 15 spectrophotometer. Measurements at 430 nm on the reduction of *o*-chloranil by inorganic sulfite[4b] generated by the solvolysis of V catalyzed by pepsin M-I at pH 3.6 and 3.75 and catalyzed by pepsin M-II at pH 3.5 indicated that the rates of inorganic sulfite formation corresponded to those of *p*-nitrophenol release. Proceeding from more acidic to less acidic reaction conditions and monitoring *p*-nitrophenol release, the k_{cat}/K_m values for the action of the modified pepsin species on V showed ascending sigmoidal pH dependencies. The pK_a values of the ionizing groups on the enzyme which are reactive in their basic forms and which appear to be responsible for this behavior are 3.9 in the case of pepsin M-I and 4.3 for pepsin M-II, and the $(k_{cat}/K_m)_{lim}$ values are $(9.1 \pm 2.4) \times 10^2\ M^{-1}\ sec^{-1}$ and $(1.7 \pm 0.2) \times 10^3\ M^{-1}\ sec^{-1}$, respectively.

In contrast to the observations with the modified pepsin species, native pepsin shows marked enantiomeric specificity when it reacts with racemic IV and V. Over a considerable acidic pH range, with the native enzyme in excess, observation of the release of *p*-nitrophenol reveals that the rates of hydrolysis of these compounds show biphasic character. The rate data can be analyzed in terms of a fast and a slow reaction, both following pseudo-first-order rate laws and accounting, respectively, for 50% of the decomposition of the ester. Measurements on the hydrolysis of racemic V at pH 4.0 (acetate buffer, $\mu = 0.05$), for instance, using enzyme concentrations ranging from 2×10^{-4} to $1.1 \times 10^{-3}\ M$ gave values of $k_{cat}/K_m = 2.5 \times 10^3\ M^{-1}\ sec^{-1}$ for the fast reaction and $4.5 \times 10^2\ M^{-1}\ sec^{-1}$ for the slow reaction. In marked contrast to our findings on the reaction of V with modified pepsin, measurements on the variation of k_{cat}/K_m for the pepsin-catalyzed hydrolysis of the fast-reacting isomer of V indicated that, as in the enzymatic hydrolysis of III,[4a] this parameter decreases at the higher pH values.[10]

While sequencing information concerning the site in pepsin which reacts with I is incomplete, the stoichiometric modification of the enzyme by several other diazocarbonyl compounds has been determined to occur at the β-carboxyl group of an active site aspartate residue[11-13] in the sequence Ile-Val-Asp-Thr-Gly-Thr-Ser-Leu, and it seems reasonable to assume that this is the residue with which I also reacts.[14] A different active site aspartate group which is in the sequence Ile-Phe-Asp-Thr-Gly-Ser-Ser-Asn is modified by II (revised sequence, privately communicated to us by Professor J. Tang).

(10) In the case of the native pepsin-catalyzed hydrolysis of III, it was found that the values of k_{cat}/K_m decreased when an enzyme-bound group with pK_a = 5.2 lost a proton.[4a]

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(14) Professor B. F. Erlanger has informed us (private communication) that two peptide fragments containing the aspartate residue which reacts with I have been isolated. In addition to aspartate, one includes isoleucine and valine, and the other contains threonine and valine. These observations are consistent with the assignment of the site modified as the aspartate group which reacts with other diazocarbonyl compounds.[11-13]

Our results with pepsin M-I and pepsin M-II demonstrate that neither of the two aspartate β-carboxyl groups in pepsin which are believed on the basis of the chemical modification data to be important active site residues in the enzyme's peptidase action is essential to the catalytic action of the enzyme on sulfite ester substrates. The k_{cat}/K_m–pH profiles obtained with pepsin M-I and pepsin M-II suggest strongly that only a single active site carboxylate group is necessary for the enzymatic hydrolysis of sulfite esters.[15] Furthermore, although the k_{cat}/K_m–pH dependencies for the action of the modified pepsins and of pepsin itself on V are quite different, the $(k_{cat}/K_m)_{lim}$ values found in our work with the former species lie between the values obtained for the hydrolysis of the fast- and slow-reacting enantiomers of V catalyzed by the native enzyme.

A reasonable interpretation of our observations on the action of modified pepsins on sulfite esters is that the carboxylate group required for pepsin's sulfite esterase activity differs from those necessary for peptidase activity in spite of the evidence that the active sites for the two types of activities overlap. It must be pointed out, however, that the elimination of peptidase activity by the esterification of either of the aspartate β-carboxyl groups modified in pepsin by I or by II does not constitute positive proof that these groups in their unmodified forms are catalytic participants in the peptidase action of the enzyme. The possibility remains that although esterification of these carboxylate functions blocks the peptidase and not the sulfite esterase action, the carboxylate group crucial to the hydrolysis of sulfite esters is one of those required for the action of the enzyme on peptides.[16] Further studies which should clarify the interpretation and the implications of our discovery that pepsin M-I and pepsin M-II can function as catalytically active species are in progress in our laboratory.

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(15) Despite the apparent dependence of the rate parameter k_{cat}/K_m for the pepsin-catalyzed hydrolysis of bis-*p*-nitrophenyl sulfite (III) on the state of ionization of two carboxyls in the native enzyme,[4] only one of these may be directly involved in the catalytic process while the ionization of the other could be reflected in the kinetics of reaction primarily because of its inhibitory effect on catalysis.

(16) If this postulate were correct, then the differences between the k_{cat}/K_m–pH profiles for the action of pepsin M-I and pepsin M-II on V and the action of the native enzyme on a variety of substrates (J. S. Fruton in "The Enzymes," Vol. III, 3rd ed, P. D. Boyer, Ed., Academic Press, New York, N. Y., 1971, pp 120–164) would indicate that esterification of a carboxyl group in the vicinity of the active site causes a substantial alteration in the pK_a for the ionization of the catalytically active carboxylate function.

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Reactions of Tungsten and Molybdenum Atoms with 1,3-Butadiene. Tris(butadiene)tungsten and -molybdenum

Sir:

In studying the reactions of atomic tungsten and atomic molybdenum in the condensed phase, we have found that they react readily with a variety of olefinic

and aromatic reagents. We would like to report here the adducts with 1,3-butadiene since they appear to be unique in the literature of organometallic compounds. The products are tris(butadiene)tungsten(0) and tris(butadiene)molybdenum(0).

Tungsten and molybdenum atoms are generated by resistive heating of the respective wires, employing essentially the same apparatus and conditions as for previous reports of metal atom reactions.[1] Cocondensation of the atoms with 1,3-butadiene (1:100 molar ratio) at liquid nitrogen temperature produces a yellow matrix in both instances. After warm-up the yellow-brown liquid was siphoned from the flask under an inert atmosphere and the excess 1,3-butadiene was pumped off, leaving a residue which could be purified by sublimation (10^{-3} Torr, 50–60°) or by recrystallization from heptane. Yields are 50–60% based on the amount of metal deposited in the butadiene matrix. A typical 1 hr run yields about 200 mg of pure product.

Tris(butadiene)tungsten: the pure crystalline material is white, is in the hexagonal system, decomposes at 135°,[2] is soluble in organic solvents, and is stable in air (slight darkening noticeable after 2 weeks). The infrared spectrum (CCl_4) showed complexed carbon–carbon double bond stretching frequencies at 1485 (vw) and 1440 cm^{-1}(w). The ^1H nmr (C_6D_6) exhibited multiplets at τ 5.72, 8.50, and 9.62, of equal intensities. The following major ions with their respective masses were observed (70 eV, 75°): $(C_4H_6)_3W^+$ (*m/e* 346, relative intensity 85%), $(C_4H_6)_2W^+$ (*m/e* 292, relative intensity 100%). The high resolution mass spectrum provided the following mass for the ^{186}W isotope: calcd, 348.0951; found, 348.0959.

Tris(butadiene)molybdenum: the pure crystalline material is yellow, is in the hexagonal system, decomposes at 130°,[2] is stable in air (darkening after 1 week), and is soluble in organic solvents. Complexed carbon–carbon double bond stretching appeared at 1437 cm^{-1} (w, CCl_4). The ^1H nmr showed multiplets at τ 5.27, 8.32, and 9.46 of equal intensities. These major ions with their respective masses were observed (70 eV, 75°): $(C_4H_6)_3Mo^+$ (*m/e* 260, relative intensity 41%), $(C_4H_6)_2Mo^+$ (*m/e* 206, relative intensity 50%), $(C_4H_6)Mo^+$ (*m/e* 152, relative intensity 19%). The high resolution mass spectrum provided the following mass for the ^{98}Mo isotope: calcd, 260.0463; found, 260.0450.

While there have been many attempts to stabilize tungsten(0) and molybdenum(0) with ligands other than C≡O,[3-6] these have usually resulted in only partial substitution or at best difficult and extended reactions. King[4,5] has reported that substitution reactions with $(CH_3CN)M(CO)_3$ (M = Mo, W) can be carried out. In only one case, however, was he able to obtain complete substitution, that of methyl vinyl ketone. He found that cyclic conjugated dienes would only replace some of the ligands while butadiene would not react at all. Fischer *et al.*,[7] have shown that the photochemical addition of butadiene to $Mo(CO)_6$ produced only bis(butadiene) $Mo(CO)_2$ after extended irradiation. The addition of metal atoms to organic substrates offers a useful route to organomolybdenum and tungsten complexes previously unobtainable by conventional routes.

An octahedral symmetry characteristic of the group VI transition metals is presumed for both complexes. The crystallographic structure[8] of the related tris(methyl vinyl ketone)tungsten has recently been shown to be the unusual trigonal prismatic type. X-Ray studies are presently underway in this laboratory to determine the structure of these tris(diene)metal(0) complexes.

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Bis(1,4-cyclohexadiyl)-1,2-dioxetane and Bis(1,4-cyclohexadiyl)oxirane from Photooxidation of 7,7′-Binorbornylidene

Sir:

2,2′-Biadamantylidene (**8**) has become a compound of particular interest since its photooxidation affords a dioxetane of unusual stability.[1] Recently, the photooxidation of **8** in pinacolone[2a] has been interpreted as indicating the stripping of a perepoxide intermediate[2b] by a Baeyer–Villiger process.[2c] In view of the increasing interest in hindered alkenes of this type, we undertook the synthesis of 7,7′-binorbornylidene (**3**). The preparative method used for **3** also provided a way of making **8** in improved yield.

A solution of 2.91 g of 7-norbornanone (**1**)[3] in 15 ml of petroleum ether was stirred with 22.9 g of phosphorus(V) bromide[4] and heated to 70° for 40 hr. The work-up procedures are identical with those for 2,2-dibromoadamantane.[5a] Recrystallization from petroleum ether gave 5.63 g (84%) of 7,7-dibromonorbornane (**2**): mp 89–90°; ir (KBr) 2940, 1450, 1300, 1180, 1150, 1020, 988, 952, 888, 876, 825, and 760 cm^{-1}; nmr ($CDCl_3$)[6] δ 1.10–1.50 (m, 4 H, endo), 1.90–2.30 (m, 4 H, exo), 2.40–2.60 (m, 2 H, bridgehead); mass spectrum *m/e* 256, 254, and 252 (P, 1:2:1); 214, 212, and 210 (1:2:1); 175 and 173 (base, 1:1).

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