clude that the splitting is quite similar throughout the series, with perhaps a slight tendency to increase as the NNC angle increases.

Indirect support for our assignment of the pes of **1**(1) can be obtained by noting that in a certain sense our assignments appear to be consistent with the pes of 2,3-diazabicyclo[2.2.1]heptane.[6] Upon forming the hydrazine derivatives of **1**(*n*), the N–N bond would be expected to lengthen and the dihedral angle between the "lone pair" orbitals would increase from 0 to about 120°. Consequently the interaction of the "lone pair" orbitals would be less. This is, in fact, confirmed by the pes of the hydrazine derivative of **1**(1) which indicates a value of 1.81 eV for Δ^6 compared with the value of 2.95 eV reported here for **1**(1).

Because of its low extinction coefficient, the observed uv transition of **1**(*n*) (*cf.* Table II) is certainly of the $n \rightarrow \pi^*$ type. Recent *ab initio* calculations[16] indicate that for cis azo compounds the $n \rightarrow \pi^*$ transition energy decreases as $\angle NNC$ increases, mainly because the n_- level is destabilized while the π^* level remains approximately unchanged. Thus within a series of closely related molecules we can expect that the variation of IP_1 parallels the variation of the $n \rightarrow \pi^*$ transition energy. This is indeed the case for **1**(*n*) for which a reasonable correlation of λ_{max} (in eV) *vs.* IP_1 is observed (*cf.* Tables I and II); there is a small discrepancy for **1**(4) but slight changes in correlation energy and orbital reorganization effects prevent a strictly quantitative correlation between λ_{max} and IP_1.

Finally we would like to point out an interesting feature of the pes of all cis azo molecules that have been studied to date; the separation of the n_- and π levels is remarkably constant (about 2.5 ± 0.2 eV) whereas the splitting of the n_+ and n_- "lone pair" orbitals can be as small as 1.6 eV in 3,4-diazatricyclo[4.2.1.$0^{2,5}$]non-3-ene[15] or as large as 3.5 eV in diazirine.[2]

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(16) N. C. Baird, P. de Mayo, J. R. Swenson, and M. C. Usselman, *Chem. Commun.*, 314 (1973); for a discussion of the uv spectra of azo compounds see also H. Rau, *Angew. Chem., Int. Ed. Engl.*, **12**, 224 (1973).

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Configuration of the β-Carbon Atoms of the β-Methyllanthionine Residues in Nisin

Sir:

β-Methyllanthionine occurs in yeast[1] and as a constituent of several interesting heterodetic polycyclic peptides, the structures of two of which have recently been elucidated.[2,3] Although the configurations of alanine and aminobutyric acid obtained after desulfurization have been determined,[2,3] the assignment at the β-carbon atom had to await comparison of the natural material with synthetic β-methyllanthionine of known configuration.

This has now been accomplished for the isomer occurring in nisin,[2] and several observations have been made pertaining to the problem in general.

Starting materials for the synthesis of the isomers of β-methyllanthionine are the diastereoisomeric pairs A and B of DL-β-methyl-*S*-benzylcysteine prepared by the addition of benzylmercaptan to the azlactone of benzoyldehydrobutyrine followed by fractional crystallization and hydrolysis of the products.[4] The configurational identities were determined by application of the cyanogen bromide reaction[5] to the *N*-acetyl-β-methyl-*S*-methylcysteines. The benzyl group was removed by reaction with anhydrous hydrogen fluoride (HF) for 1 hr at room temperature in the presence of anisole. After evaporation of the HF and washing with ethyl acetate, the amino acids were *S*-methylated by treatment with methyl-*p*-nitrobenzene sulfonate in 0.05 *M* phosphate buffer (pH 8.5) under nitrogen. The *S*-methyl derivatives were treated with a fourfold excess of acetic anhydride at the same pH, and sufficient 88% formic acid was added to give a 60% formic acid solution. Exposure to 3 equiv of cyanogen bromide at room temperature for 2 days resulted in the transformation of 50% of the acetyl-DL-β-methyl-*S*-methylcysteine A to 97% pure DL-*O*-acetyl-*allo*-threonine (Figure 1) while the amino acids B, correspondingly yielded DL-*O*-acetylthreonine of identical configurational purity.

The identity of the products was determined by comparison of their elution volumes from cation exchange resin columns (0.9 × 60 cm, 53°, 0.2 *N* sodium citrate buffer, pH 3.25) with those of authentic samples prepared from DL-threonine and DL-*allo*-threonine by treatment with 1 *N* HCl in glacial acetic acid and crystallization from alcohol–ether. Since an inversion of configuration at the β-carbon atom is expected in the cyanogen bromide reaction (Figure 1), the pair designated A must be the DL-threo amino acids, while B are the DL-allo isomers.

The β-methyllanthionines were obtained from the reaction of the β-methylcysteines with *N*-formyl-L-β-chloroalanine.[6] The latter was prepared by dissolving L-chloroalanine hydrochloride in anhydrous formic acid, adding 1 equiv of sodium bicarbonate and 20 equiv of preformed formic acetic anhydride[7] at 10° in two portions separated by a 2-hr interval. The mixture was lyophilized after 4 hr and the product allowed to react with the thiol amino acids in 0.05 *M* phosphate buffer (pH 8.5) under nitrogen. The formyl group was removed with 6 *N* HCl in 10 min at 100°.[8] Amino

(1) P. Downey and S. Black, *J. Biol. Chem.*, **228**, 171 (1957).

(2) E. Gross and J. L. Morell, *J. Amer. Chem. Soc.*, **93**, 4634 (1971).

(3) E. Gross, H. H. Kiltz, and E. Nebelin, *Hoppe-Seyler's Z. Physiol. Chem.*, **354**, 810 (1973).

(4) H. E. Carter, C. M. Stevens, and L. F. Ney, *J. Biol. Chem.*, **139**, 247 (1941).

(5) E. Gross, *Methods Enzymol.*, **11**, 238 (1966).

(6) β-Chloroalanine not protected at the amino group reacts *via* aziridine intermediates to generate undesired products resulting from the addition of sulfhydryl groups to the α-carbon atom.

(7) V. C. Mehlenbacher, *Org. Anal.*, **1**, 37 (1953).

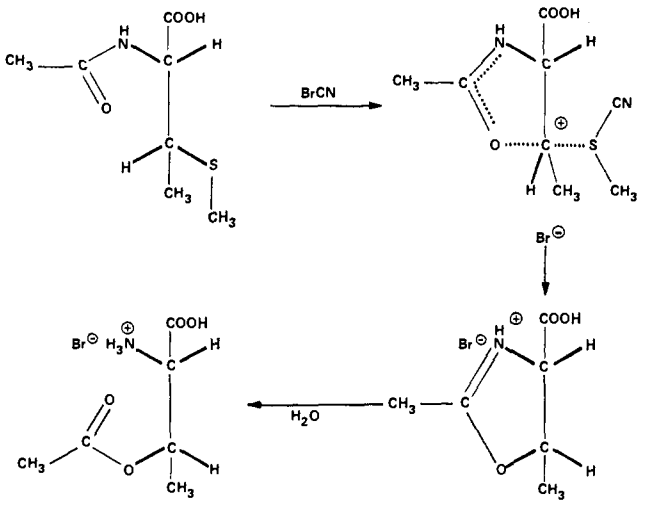

Figure 1. The reaction of *N*-acetyl-β-methyl-*S*-methylcysteine with cyanogen bromide.

acid analysis indicated an initial 5% per day rate of formation of β-methyllanthionine. The only side reaction of significance, the formation of serine, proceeded at half that rate. The products from DL-*threo*-β-methylcysteine are not separated in the chromatographic system employed (peak II, Figure 2); those from DL-*allo*-β-methylcysteine are eluted at different effluent volumes (peaks I and I′, Figure 2).[9]

The isomer of β-methyllanthionine occurring in nisin[2] coelutes with the threo derivatives and must therefore have the L-*configuration at the β-carbon atom* since the α-carbon atom of the amino butyric acid moiety had previously been assigned the D configuration.[2]

Inasmuch as the assignment of configuration is based on properties of the amino acids obtained by acid hydrolysis, knowledge of the extent of racemization occurring under these conditions is necessary. Lanthionine is completely racemized under the conditions of acid hydrolysis.[2] Isolation of the isomers represented by peak I′ and treatment with 6 *N* HCl for 24 hr at 110° results in the transformation of 30% of the material to the isomers of peak I and 10% to the isomers of peak II. When the isomers of peak II are exposed to the same conditions, they are transformed to the extent of 10% to the isomers of peaks I and I′.

Evidently the α-carbon atom of the alanine moiety is subject to more extensive configurational change than that of the amino butyric acid portion. This is consistent with the occurrence of 0.5 residues of *allo*-β-methyllanthionine in hydrolysates of nisin which contains a total of four residues of the amino acid. Thus, only 63% of the amino acid in a hydrolysate retains the original configurations, while 27% is inverted at the alanine moiety alone, 3% at both the α-carbon atoms of the alanine and amino butyric acid portions, and 7% at the α-carbon atom of the amino butyric acid moiety only. In spite of this, β-methyllanthionine having the correct configuration at the α-carbon atoms[3]

(8) This procedure gave 95% pure L-lanthionine in a trial synthesis wherein the undesired product of epimerization (*meso*-lanthionine) is easily detected by ion exchange chromatography.

(9) In a synthesis involving addition of DL-cysteine to benzoylidehydrobutyrineazlactone, the quantities of the isomers of peaks I, II, and I′ are in the proportion 1 : 2 : 1.

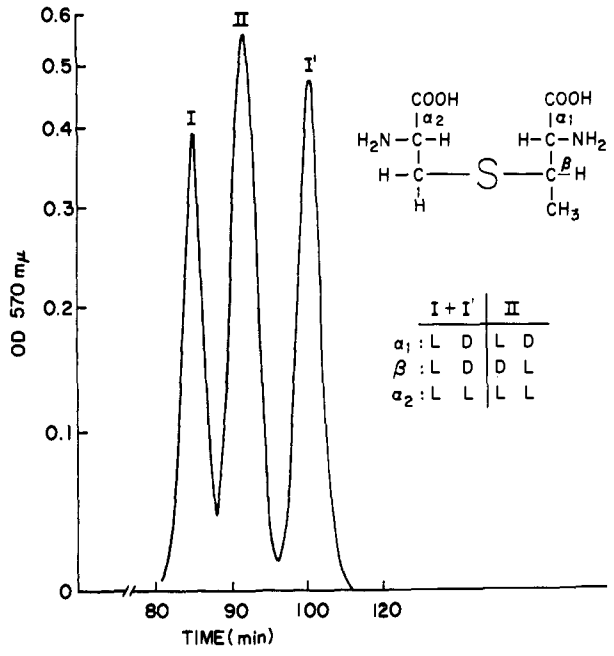

Figure 2. The separation of the isomers of β-methyllanthionine by ion exchange chromatography (column: 0.9 × 60 cm, 53°, 0.2 *N* sodium citrate buffer, pH 3.25). Isomers not shown in inset are enantiomers with the D-alanine moiety.

and showing the elution behavior of the threo compound[10] was isolated by the selective process of crystallization from a hydrolysate of subtilin.[11]

(10) A. Schöberl, E. Gross, J. L. Morell, and B. Witkop, *Biochim. Biophys. Acta*, **121**, 406 (1966).

(11) G. Alderton, *J. Amer. Chem. Soc.*, **75**, 2391 (1953).

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$Fe[OAl(O\text{-}n\text{-}Bu)_2]_2$. A New Molecular Oxygen Activator

Sir:

We want to report the behavior of an oxo alkoxide of aluminum and iron(II) as a reversible molecular oxygen activator.

This amorphous compound, highly soluble in hydrocarbons, is obtained by a straight condensation reaction (at 500°K in decalin under argon atmosphere), according to eq 1. Its composition was established by

$$2Al(OR)_3 + Fe^{II}(CH_3COO)_2 \longrightarrow (OR)_2AlOFeOAl(OR)_2 + 2CH_3COOR \quad (1)$$

elementary analysis;[1] the Al/Fe ratio is found to be near 1.98 by complexometric titration (EDTA in acetate buffer after oxidation of Fe(II) into Fe(III)). The OR/Al ratio as determined by glc after hydrolysis of the complex equals 1.96. All these values are consistent with the expected ones for eq 1, *i.e.*, Al/Fe = OR/Al = 2. R may be a *n*-butyl, isopropyl, or isobutyl group. Other oxo alkoxides may also be prepared in the same

(1) M. Osgan and Ph. Teyssié, *J. Polym. Sci., Part B*, **5**, 789 (1967); T. Ouhadi, A. J. Hubert, and Ph. Teyssié, to be submitted for publication.