and simple repeated washing of the remaining solids with methanol which completely removed excess reagents and by-products.[8]

For a typical experiment insulin (92 mg) was dissolved in dry liquid ammonia (300 ml) which was stirred under nitrogen and protected from moisture and CO_2. Dithiothreitol (148 mg, corresponding to 20 mol of DTT/mol of disulfide) was added. The solution was kept at the boiling point (at about −33°, atmospheric pressure) for 1 hr. Chloromethane gas was then introduced until the thiols were fully methylated (after 1–3 min). Complete *S*-alkylation was ascertained by a negative nitroprusside reaction directly in the liquid ammonia solution[6] of aliquots. The liquid ammonia evaporated spontaneously. The vacuum-dried residue was triturated with methanol (15 ml), centrifuged, and washed three–four times with methanol (10 ml each) and finally with peroxide-free ether, followed by centrifugation. A white powder was obtained after drying (95 mg, 98%, mixture of tetra-*S*-methyl A chain and bis-*S*-methyl B chain[9]). In other preparations the methanol-wet pellet was dissolved in 0.1 *N* ammonium hydroxide or in 50% acetic acid and lyophilized. Amino acid analysis[10] gave: *S*-methylcysteine 6.2, no trace of cysteine or cystine, and values for all other amino acids (based on Ala, 3.0) which agreed very closely with the theory.

The above example shows the efficiency and convenience of the procedure, because (a) complete and selective *S*-methylation of peptides and proteins has until recently been unattainable,[11] (b) complete reduction and *S*-alkylation of insulin previously has been difficult,[4] and (c) salt-free, fully *S*-alkylated protein derivatives are obtained within 1 day in close to quantitative yields. Sodium borohydride, mercaptoethanol, and dithiothreitol were examined for their usefulness in the liquid ammonia procedure. Dithiothreitol gave the best results. A 20-fold molar excess of DTT per mole of disulfide was generally sufficient. To assure full conversion of disulfides to thiols, 1-hr reduction periods were usually applied.

Thiol alkylation was best achieved by addition of alkyl chlorides directly to the liquid ammonia solution.[6,7] Alkyl iodides and bromides, commonly used for *S*-alkylation in aqueous solution,[4] reacted to considerable degrees with liquid ammonia, but alkyl chlorides appeared to be practically inert under the conditions employed in this procedure.[12] To assure complete *S*-alkylation, a 20-fold molar excess of alkyl chloride over dithiothreitol was used. Liquid alkylating agents (*e.g.*, benzyl chloride) were conveniently added by pipet, solid reagents (chloroacetic acid, chloroacetamide, β-chloroethylamine hydrochloride) through a powder funnel. The following products were prepared in 90–98% yields essentially as described above:[13] (bis-*S*-methyl)lysine-vasopressin [*S*-methylcysteine, 2.1 (2)]; (hexa-*S*-benzyl)insulin [*S*-benzylcysteine, 5.9 (6)]; (tetra-*S*-benzyl)neocarzinostatin[14] [*S*-benzylcysteine, 3.8 (4)]; (tetra-*S*-carboxymethyl)-neocarzinostatin [*S*-carboxymethylcysteine, 3.9 (4)]; (octa-*S*-carboxamidomethyl)lysozyme [*S*-carboxymethylcysteine, 7.8 (8)]; (oct-*S*-β-aminoethyl)lysozyme [*S*-β-aminoethylcysteine, 7.6 (8)]. To ascertain the absence of undesired peptide bond cleavage, N-terminal analysis was carried out using 1-dimethylaminonaphthaline-5-sulfonyl chloride.[15] Identical end groups were obtained from starting native proteins and reduced and *S*-alkylated products.

In conclusion, full reduction of disulfide bonds and complete and selective *S*-alkylation of liquid ammonia–soluble peptides and proteins[16] are described. This procedure offers an alternative approach for the modification of proteins possessing disulfides that resist reduction in aqueous solution. Uniform derivatives, potentially useful in many areas of protein chemistry, can be prepared effectively and rapidly. Principal improvements over the previously known sodium in liquid ammonia procedure[5–7] consist in (a) absence of peptide bond scission or other side reactions, and (b) complete removal of reagents and by-products by simple washing with methanol.

(8) Complete removal of buffer salts, denaturants, reducing agents (*e.g.*, $NaBH_4$), and alkylating agents after disulfide reductions in aqueous phase requires laborious procedures, such as dialysis, gel filtration, or ion exchange chromatography; see ref 4.

(9) Thin-layer chromatography (50% acetic acid) of the isolated product gave two spots (A and B chains).

(10) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, 30, 1190 (1958); D. H. Spackman, *Methods Enzymol.*, 11, 3 (1967).

(11) The use of methyl *p*-nitrobenzenesulfonate in aqueous solution [R. L. Heinrikson, *Biochem. Biophys. Res. Commun.*, 41, 967 (1970)] still requires time-consuming work-up (dialysis); see ref 4. *S*-Methylated polypeptides are of interest since chemical cleavage at *S*-methylcysteine residues has been described [E. Gross, J. L. Morrell, and P. Q. Lee, *Proc. Int. Congr. Biochem.*, 7*th*, Part XI, 535 (1967); T. F. Spande, B. Witkop, Y. Degani, and A. Patchornik, *Advan. Protein Chem.*, 24, 119 (1970)].

(12) No glycine could be detected by amino acid analysis in a *S*-carboxymethylated product prepared with the use of chloroacetic acid; in contrast, large amounts of glycine resulted from the use of iodoacetic acid. However, no potential side reactions of alkyl iodides with lysine, histidine, methionine, or tryptophan residues (see Bailey, ref 4) were detected by amino acid analysis or by ultraviolet difference spectrum.

(13) Degrees of reduction and alkylation were determined from the amounts of *S*-alkylcysteine (given in brackets, theory in parentheses) in amino acid analyses after total acid hydrolysis.

(14) H. Maeda, K. Kumagai, and N. Ishida, *J. Antibiot., Ser. A*, 19, 253 (1966); J. Meienhofer, H. Maeda, C. B. Glaser, and J. Czombos, "Proceedings of the Second American Peptide Symposium, Cleveland, Ohio, 1970," S. Lande, Ed., Gordon and Breach, New York, N. Y., in press.

(15) W. R. Gray, *Methods Enzymol.*, 11, 139 (1967); K. R. Woods and K. T. Wang, *Biochim. Biophys. Acta*, 133, 369 (1967).

(16) For proteins that do not dissolve in liquid ammonia the procedure should be modified. For instance, human growth hormone and bovine pancreatic ribonuclease A are insoluble. The former was completely reduced in suspension using 1000-fold excess of DTT and then quantitatively benzylated. Alternatively, it was dissolved in a very small volume of water (10 mg in 0.5 ml) and then added to liquid ammonia (100 ml) where it remained in solution. The ribonuclease dissolved slowly in the presence of 20 equiv of DDT within 4–5 hr. Addition of alkylating agents at this point gave 4-*S*-alkylcysteines.

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Trimethylenemethane and the Methylenecyclopropane Rearrangement[1]

Sir:

There has been much interest recently in the methylenecyclopropane rearrangement,[2] and in the nature of trimethylenemethane (**1**) which has been prepared[3] as an observable entity in matrices at low temperatures.

(1) This work was supported by the Air Force Office of Scientific Research through Contract No. F44620-C-70-0121.

(2) For a summary and references, see W. von E. Doering and H. D. Roth, *Tetrahedron*, 26, 2825 (1970).

(3) P. Dowd, *J. Amer. Chem. Soc.*, 88, 2587 (1966); 89, 715 (1967).

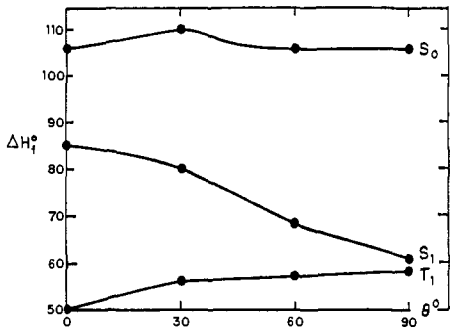
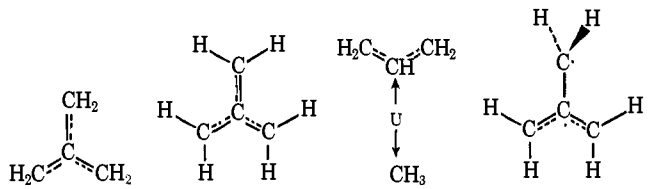


Figure 1. Dependence of heat of formation on twist angle.

We wish to report some calculations that have a bearing on both these problems.



1 2 3 4

Previous theoretical studies[4] and experiments[3] agree in concluding that **1** has a triplet ground state with the symmetrical structure indicated in **2** and it seems to have been generally assumed that the singlet forms of **1** must be likewise planar. The following intuitive argument suggested to us that this might not in fact be so. One can derive **1** by union[5] of allyl and methyl radicals as indicated in **3**. In the HMO approximation, the odd electron in allyl occupies a MO confined to the terminal atoms, the central atom carrying no unpaired spin. In the SCF approach, however, the high concentration of, say, α spin at the terminal atoms leads to an uncoupling of the other two π electrons with a consequent excess of β spin at the central atom. If then the unpaired electron at methyl also has α spin, one might expect union to involve a bonding interaction between methyl and the central allyl atom; on this basis one would expect the resulting structure, *i.e.*, the triplet form of **1**, to be coplanar. Conversely, if the unpaired electron on methyl has β spin, the methyl–allyl interaction should be antibonding; in this case the optimum structure for the resulting singlet form of **1** will be one in which the unpaired electrons of the allyl and methyl moieties are isolated from each other, *i.e.*, **4** in which the relevant orbitals are orthogonal.

Calculations were carried out for the system indicated in **4** for various angles (θ) of twist of the "methyl" carbon out of coplanarity with allyl. Thus $\theta = 0$ corresponds to **2** while $\theta = 90°$ corresponds to **4**. The calculations were carried out for (a) the closed shell singlet structure (S_0), (b) the triplet (T_1), and (c) an open shell singlet (S_1), in which there are two unpaired electrons of opposite spin. The calculations were carried out by the MINDO/2 method,[6,7] using the "half-electron" model[8] for b and c.[9]

Figure 1 shows plots of the calculated heats of formation (ΔH_f; kilocalories/mole at 25°) for the three structures as a function of θ. The geometry of each structure was chosen to minimize the energy using a Simplex optimization[12] introduced previously by Brown.[13]

It will be seen that the triplet is predicted to be the most stable structure and to be most stable when planar ($\theta = 0$), in agreement with experiment[3] and previous calculations.[4] However, the open shell singlet, the next most stable structure of the three, is most stable in the perpendicular form **4**. Indeed, the energy required to transform it into a planar geometry is no less than 24 kcal/mol. The energy of the closed shell structure varies with θ in a manner which at first sight seems curious, having a maximum at $\theta \simeq 30°$; this, however, can be explained in terms of two conflicting tendencies, one the tendency of singlet **1** to adopt the configuration **4**, the other stabilization of the planar form of S_0 by Jahn–Teller distortion since if it had the symmetrical planar structure **2** it would be degenerate.

These calculations therefore lead to the prediction that the ground state of singlet **1** is the open shell structure with one methylene orthogonal to the remaining atoms, *i.e.*, **4**. This then should be the form in which **1** will be generated by any process leading to a singlet configuration.

The degenerate rearrangement of methylenecyclopropane and analogous rearrangements of its derivatives were originally thought to take place *via* **1** which was assumed to have the symmetrical planar structure **2**. On this basis it is impossible to explain the retention[2] of optical activity during rearrangement of Feist's ester **5** to a mixture of **6** and **7**. Doering and Roth[2] deduced that the reaction must either be concerted or must take place *via* a nonplanar form of **1** with the geometry indicated in **4** and a similar intermediate has been postulated[14] in the rearrangement of 2,2-diphenylmethylenecyclopropane. Our calculations are of course entirely consistent with the oc-

(4) Y. Gondo and A. H. Maki, *J. Chem. Phys.*, **50**, 3638 (1969), and references cited in ref 2 for earlier calculations.

(5) M. J. S. Dewar, "The Molecular Orbital Theory of Organic Chemistry," McGraw-Hill, New York, N. Y., 1969, Chapter 6.

(6) M. J. S. Dewar and E. Haselbach, *J. Amer. Chem. Soc.*, **92**, 590 (1970).

(7) N. Bodor, M. J. S. Dewar, A. Harget, and E. Haselbach, *ibid.*, **92**, 3854 (1970).

(8) M. J. S. Dewar, J. A. Hashmall, and C. G. Venier, *ibid.*, **90**, 1953 (1968); M. J. S. Dewar and N. Trinajstić, *Chem. Commun.*, 646 (1970).

(9) The MINDO/2 method has been shown[6,7,10] to give good estimates of heats of atomization for a wide variety of organic compounds, including ions, radicals, and triplet states. It has also given good estimates of activation energies for a number of reactions,[11] including isomerization[6,7] of olefins and cumulenes by rotation about C=C bonds. There is therefore reason to believe that its use in the present connection should lead to reasonably trustworthy results.

(10) N. Bodor, M. J. S. Dewar, and S. D. Worley, *J. Amer. Chem. Soc.*, **92**, 19 (1970); M. J. S. Dewar, E. Haselbach, and S. D. Worley, *Proc. Roy. Soc. Ser. A*, **315**, 431 (1970); M. J. S. Dewar, E. Haselbach, and M. Shanshal, *Angew. Chem., Int. Ed. Engl.*, **9**, 738 (1970); unpublished work by N. Bodor, W. W. Schoeller, and J. S. Wasson.

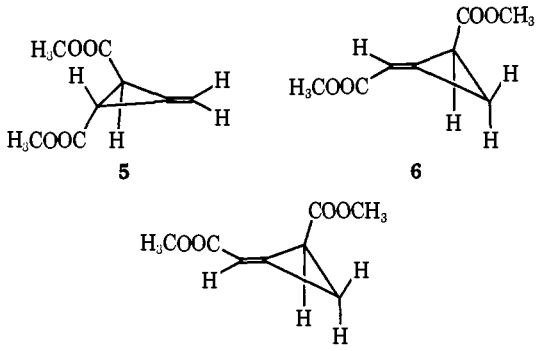
(11) M. J. S. Dewar, A. Harget, and E. Haselbach, *J. Amer. Chem. Soc.*, **91**, 7521 (1969); M. J. S. Dewar, E. Haselbach, and M. Shanshal, *ibid.*, **92**, 3505 (1970); A. Brown, M. J. S. Dewar, and W. W. Schoeller, *ibid.*, **92**, 5516 (1970); unpublished work by S. Kirschner, M. Kohn, W. W. Schoeller, and P. Weiner.

(12) J. A. Helder and R. Mead, *Comput. J.*, 7, 308 (1964); we have since modified this method by interrupting the search as the minimum is approached and building up a new simplex from *computed* steepest descent step sizes for valence force coordinates thereby avoiding false minima and often speeding up the search. This modification also makes it easy to adapt the program for nonlinear least squares.

(13) A. Brown, M. J. S. Dewar, and W. W. Schoeller, *J. Amer. Chem. Soc.*, **92**, 5516 (1970).

(14) M. Jones, Jr., M. E. Hendrick, J. C. Gilbert, and J. R. Butler, *Tetrahedron Lett.*, 845 (1970).

currence of **4** as a stable intermediate in the reaction, provided that it has the open shell singlet structure (S_1). On this basis the predominant formation of **6** rather than **7** from **5** must presumably be attributed to steric effects, because the transition states leading from **5** to **6** or **7** should be equally favorable on electronic grounds, both being nonaromatic.[6,15]



5 **6** **7**

The next step will be a detailed study of the reaction path leading from methylenecyclopropane to **2** and the effect of substituents on it. However, the results reported here seem sufficient to make any concerted mechanism unlikely, as would be expected since the geometry of the intermediate **2** is such as to inhibit effective overlap between the atomic orbitals of the methylene groups.

(15) M. J. S. Dewar, *Angew. Chem.*, in press.

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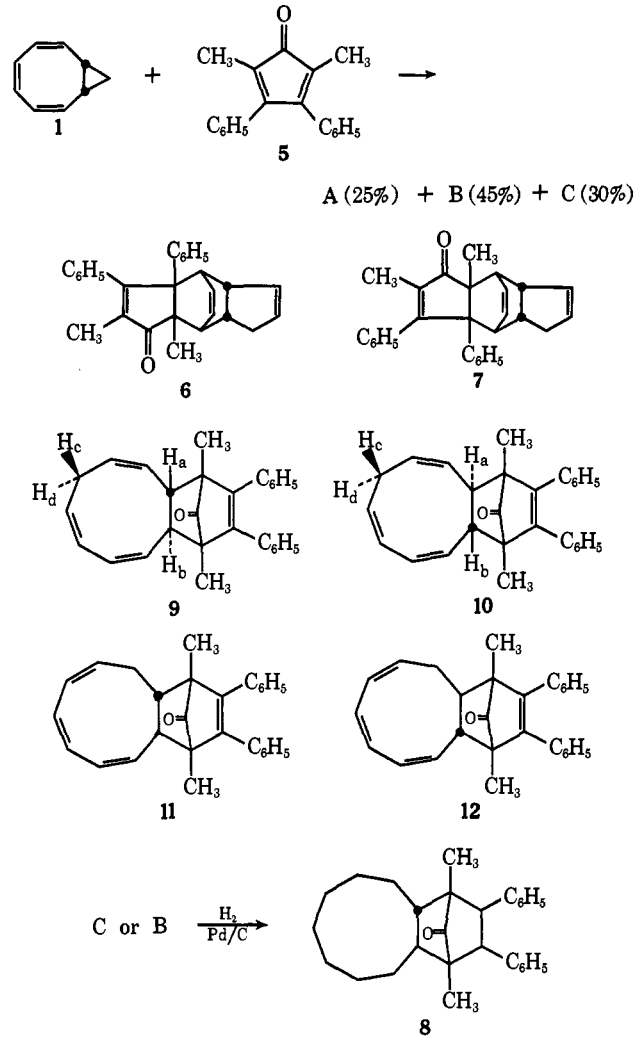
Evidence for *cis,cis,trans,cis*-1,3,5,7-Cyclononatetraene in the Thermal Bond Relocation of *cis*-Bicyclo[6.1.0]nona-2,4,6-triene

Sir:

The details of the thermal reorganization of *cis*-bicyclo[6.1.0]nona-2,4,6-triene (**1**) to *cis*-8,9-dihydroindene (**2**)[1] have been the subject of intense activity.[2-6] Concern over the nature of this rearrangement derives chiefly from the fact that while disallowed on the basis of orbital symmetry, its activation requires only mild heating. It was pointed out,[3] for example, that thermal rupture of the cross-link in **1** should occur conrotatorily to generate *cis,cis,cis,trans*-1,3,5,7-cyclononatetraene (*cis*[3],*trans*-CNT) (**3**) which, in turn, ought to electrocyclize disrotatorily to trans- rather than the observed cis-fused 8,9-dihydroindene. Interestingly, this prediction was recently substantiated in full in the case of two 9,9-dialkyl derivatives of **1** both of which were shown to produce chiefly *trans*-8,9-dihydroindene skeletons on thermolysis.[4] Further, the case for strict orbital-symmetry control within these systems was strengthened considerably by the recent observation[7-10] that thermolysis of *all-cis*-CNT (**4**) does indeed lead to the expected cis-fused 8,9-dihydroindene (**2**). Presently, we record results of key trapping experiments which, we believe, have direct bearing on the mechanistic details of the thermolysis of **1**.



1 **5** **6** **7** **9** **10** **11** **12** **8**

Treatment of **1** with an equimolar quantity of 2,5-dimethyl-3,4-diphenylcyclopenta-2,4-dienone (**5**)[11] in boiling benzene led, cleanly and quantitatively, to three 1:1 adducts:[12,13] A (25%) [white needles, mp 220–220.5°; ν_{CO}^{KBr} 1675 cm^{-1}; *m/e* 378 (P$^+$; 10%); nmr (60 MHz; $CDCl_3$) multiplets at τ 2.6–3.0 (8 H), 3.4–3.7 (2 H), 3.9–4.1 (2 H), 4.4–4.7 (2 H), 6.3–8.2 (6 H), and sharp singlets at 8.30 (3 H) and 8.90 (3 H)]; B (45%) [white crystals; mp 132–133°; ν_{CO}^{KBr} 1760 cm^{-1}; *m/e* 378 (P$^+$; 23%); nmr (60 MHz; $CDCl_3$) multiplets at τ 2.7–3.2 (10 H), 3.8–4.7 (6 H), 7.0–7.8 (4 H), and sharp singlets at 8.62 (3 H) and 8.83 (3 H)]; and C (30%) [white plates; mp 127–128°; ν_{CO}^{KBr} 1760 cm^{-1}; *m/e* 378 (P$^+$; 31%); nmr (60 MHz; $CDCl_3$) multiplets

(1) E. Vogel, *Angew. Chem.*, **73**, 548 (1961); **74**, 829 (1962).
(2) W. Grimme, *Chem. Ber.*, **100**, 113 (1967).
(3) A. G. Anastassiou, *J. Amer. Chem. Soc.*, **90**, 1527 (1968).
(4) S. W. Staley and T. J. Henry, *ibid.*, **91**, 1239, 7787 (1969).
(5) P. Radlick and W. Fenical, *ibid.*, **91**, 1560 (1969).
(6) J. C. Barborak, T. -M. Su, P. v. R. Schleyer, G. Boche, and G. Schneider, *ibid.*, **93**, 279 (1971).
(7) G. Boche, H. Boehme, and D. Martens, *Angew. Chem., Int. Ed. Engl.*, **8**, 594 (1969).
(8) P. Radlick and G. Alford, *J. Amer. Chem. Soc.*, **91**, 6529 (1969).
(9) A. G. Anastassiou, V. Orfanos, and J. H. Gebrian, *Tetrahedron Lett.*, 4491 (1969).
(10) S. Masamune, P. M. Baker, and K. Hojo, *Chem. Commun.*, 1203 (1969).
(11) C. F. H. Allen and J. A. Van Allan, *J. Amer. Chem, Soc.*, **64**, 1260 (1942); **72**, 5165 (1950).
(12) The adducts are stable to prolonged heating at the reaction temperature.
(13) Correct elemental analysis was obtained for all new compounds reported.