1.1 mol of bromine in dichloromethane at −12° and subsequent treatment of the reaction mixture with 2.4 mol of potassium *tert*-butoxide and 8 mol of 1,5-diazabicyclo[5.4.0]undec-5-ene in hexamethylphosphoramide at 25° for 20 hr afforded the liquid acetylene **8**[11,16] (30%). The latter rearranged smoothly in *o*-xylene at 120° within 1 hr to the crystalline tetrahydrobenz[*c*]phenanthridine **9**[10,11] (mp 137–140°; 73%). Hydroboration of **9** with an excess of diborane in tetrahydrofuran at 25° for 1 hr, followed by oxidation of the adduct with hydrogen peroxide, produced in 68% yield a 1:1 mixture of the alcohol **10**[10,11] (mp 171–172°; nmr J_{AB} = 6 Hz, J_{BC} = 4 Hz) and its C-4b epimer[10,11] (mp 177–182°), which were separated by chromatography on silica gel. Jones oxidation[17] of the cis-fused alcohol **10** at 0° for 3 min gave the ketone **11**[10,11] (mp 172–173°; nmr J_{AB} = 2.5 Hz; 32%). Reduction of **11** with sodium borohydride in methanol–dioxane (1:1) at 0° for 1 hr proceeded stereospecifically to form exclusively the desired cis,cis alcohol **12**, R = $COOC_7H_7$[10,11] (mp 214–217°), which after hydrogenolysis of the benzyloxycarbonyl group (Pd/C, ethanol) afforded *dl*-norchelidonine **12**, R = H[11,16] (mp 212–217°; nmr J_{AB} = 3.5 Hz, J_{BC} = 2 Hz; 90%). The synthetic *dl*-norchelidonine, which exhibited the same uv, ir (CH_2Cl_2), nmr, and mass spectra and identical chromatographic behavior as the natural levorotatory alkaloid, furnished upon N-methylation[7] *dl*-chelidonine (**12**, R = CH_3), mp 217–218°, after crystallization from ethanol. The synthetic and natural *dl*-chelidonine showed no depression of their melting points upon admixture and displayed identical ir spectra (KBr) and chromatographic properties. Further modifications of this scheme to provide complete control of stereochemistry are planned.

Acknowledgment. We wish to thank Professor J. Slavík for generous samples of natural *l*-norchelidonine and *dl*-chelidonine.

(17) C. D. Djerassi, R. R. Engle, and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).

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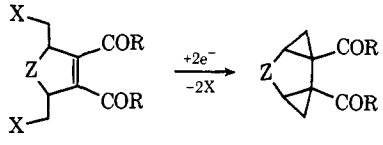
Reductive Bis Alkylation and Its Use in the Synthesis of σ-Homobenzene Derivatives[1]

Sir:

We have examined the reductive bis alkylation of 1,2-biscarbonyl-substituted ethylenes (eq 1) as an entry

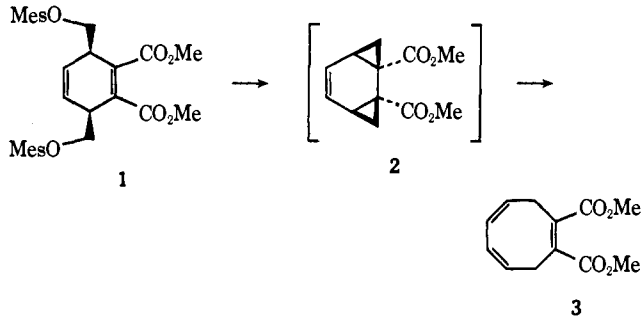


(1)

(1) We distinguish between saturated and unsaturated valence tautomers by the prefixes σ and π, respectively.

into the σ-homobenzene group of compounds with the following results.

The reaction of **1** with 2 equiv of lithium naphthalenide at −78° in tetrahydrofuran afforded on work-up a 60% yield of dimethyl 1,4,6-cyclooctatriene-1,2-dicarboxylate (**3**). Diester **3** was identified[2] by its nmr (δ 3.17 (4 H, d, J_{34} = 7 Hz, =$CHCH_2$), 3.93 (6 H, s, OCH_3), 5.8 (2 H, ABX_2 pattern, J_{AB} = 10 Hz, J_{AX} = 7 Hz, CH=$CHCH_2$), 6.25 (2 H, d, J_{45} = 10 Hz, CH=$CHCH_2$)), and conversion to cyclooctene-1,2-dicarboxylic acid[3] by sequential hydrogenation and saponification. In agreement with the mechanism proposed in eq 1, reductive bis alkylation of the dihydro derivative of **1**, **4**, afforded a material, δ 0.5 (2 H, ABX

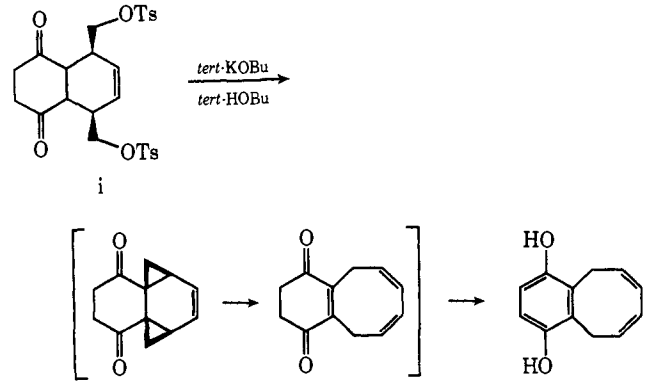


pattern, J_{AB} = 4.5 Hz, J_{AX} = 4.5 Hz), 0.8–2.2 (8 H, complex m), 3.6 (6 H, s, OCH_3), assigned structure **5**.[4]

(2) All new compounds mentioned have afforded appropriate elemental and spectral (nmr, ir, uv, mass spectral) analysis: **1**, mp 90–92°; nmr ($CDCl_3$) δ 3.02 (6 H, s), 3.6 (2 H, br), 3.8 (6 H, s), 4.35 (4 H, d, J = 5 Hz), 5.97 (2 H, d, J = 3 Hz); ir ($CHCl_3$) 5.8, 7.35, 8.5 μ; **4**, mp 89–91°; nmr ($CDCl_3$) δ 1.9 (4 H, br), 3.02, (8 H, 6 H, s superimposed on 2 H m), 3.8 (6 H, s), 4.3 (4 H, ABX pattern); ir (KBr) 5.75, 5.8 (sh), 7.4, 8.5 μ; **6**, mp 116–116.5°; nmr ($CDCl_3$) δ 0.2–1.0 (2 H, complex m), 1.2–1.6 (2 H, complex m), 1.53 (18 H, s), 3.09 (6 H, s), 3.4 (2 H, br), 4.0–4.8 (4 H, ABX pattern); ir (KBr) 5.8 (sh), 5.85, 7.4, 8.5, 8.6 μ; **9**, nmr ($CDCl_3$) δ −0.41 (1 H, m), 0.04 (1 H, m), 0.7–1.0 (2 H, m), 1.2–1.9 (3 H, m), 1.34 (9 H, s), 1.41 (9 H, s), 2.07 (3 H, s); ir (neat) 5.85 μ; **10**, mp 100.2–100.5° dec; nmr ($CDCl_3$) δ 0.0–0.7 (4 H, m), 1.0–1.9 (4 H, m), 1.41 (9 H, s), 1.45 (9 H, s), 2.5 (1 H, br), 2.98 (3 H, s), **4**.6 (2 H, ABX pattern); ir (KBr) 5.8, 5.85 (sh), 7.4, 8.6 μ; i, mp 136–138° dec; nmr ($CDCl_3$) δ 2.44 (6 H, s), 2.3–3.1 (8 H, m), 4.1 (4 H, m), 5.60 (2 H, br), 7.2–7.9 (8 H, A_2B_2 pattern); ir (KBr) 5.82, 7.4, 8.5 μ; product from i, mp 183–185°; nmr (DMSO-d_6) δ 3.5 (4 H, d, J = 4 Hz), 5.6–5.85 (4 H, br), 6.5 (2 H, s), 8.0 (2 H, br s, exchanges with D_2O); ir (KBr) 2.9 μ.

(3) J. Sicher, F. Sipos, and J. Jonas, *Collect. Czech. Chem. Commun.*, **26**, 262 (1961).

(4) A similar bis alkylation–ring expansion sequence under more usual alkylation conditions is observed when i is treated with potassium *tert*-butoxide.



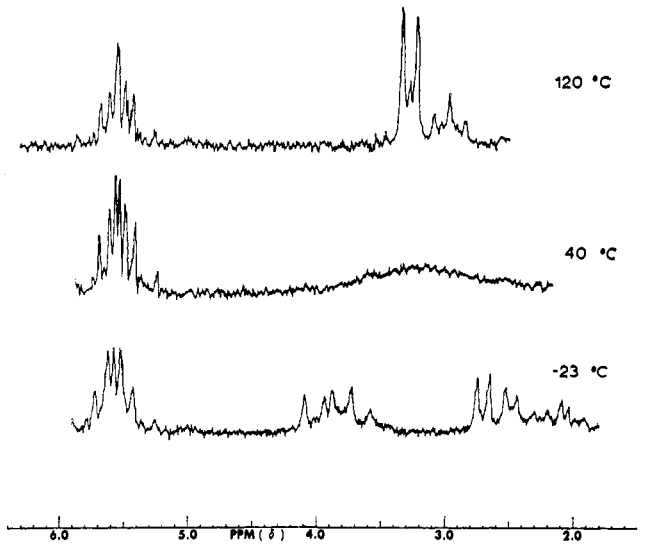
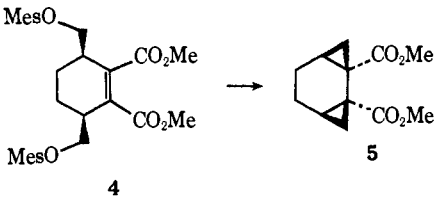
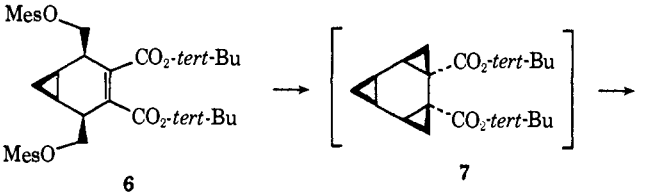


Figure 1. The temperature-dependent portion of the 60-MHz ^{1}H nmr spectrum of di-*tert*-butyl 1,4,7-cyclononatriene-1,2-dicarboxylate (**8**).

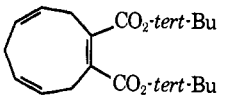


4 **5**

Reduction of **6** with 2 equiv of sodium naphthalenide at −78° afforded on work-up a 74% yield of di-*tert*-butyl 1,4,7-cyclononatriene-1,2-dicarboxylate (**8**). As required[5-8] by its structure, **8** exhibits a temperature-dependent nmr spectrum (Figure 1) that is interpretable in the high-temperature limit in terms of the time-averaged planar conformation of the cyclononatriene ring, δ 1.5 (18 H, s, OC(CH_3)), 2.97 (2 H, t, J = 7 Hz, =CH-CH_2CH=), 3.28 (4 H, d, J = 6.5 Hz, =CHCH_2), 5.57 (4 H, m, olefinic H). The structure of **8** was confirmed by sequential hydrogenation (to a tetrahydro derivative) and ozonization to azelaic acid.[9]

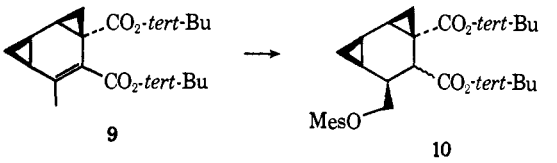


6 **7**



8

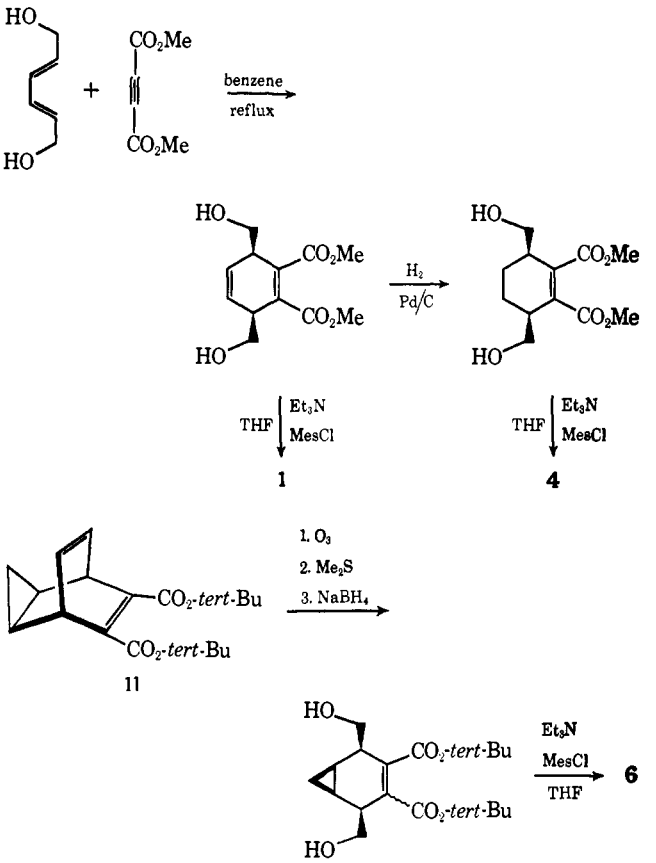
Two minor products, **9** and **10**, representing elimination and protonation of singly alkylated intermediates were also isolated. Details as to their mode of formation are unclear.



9 **10**

The syntheses of methanesulfonates **1**, **4**, and **6** are outlined in Scheme I.[10]

Scheme I



The isolated compounds **3** and **8** are believed to be derived from the σ-homobenzenes **2** and **7**, respectively, by Woodward–Hoffmann thermally allowed cycloreversions.[13] The facile cleavage of the σ bonds in the case **2** → **3** is a $-[{}_\pi 4_s + {}_\pi 2_s]$ process (retrograde Diels–Alder reaction[14]) and in the case **7** → **8** is a $-[{}_\pi 2_s + {}_\pi 2_s + {}_\pi 2_s]$ process (retrograde homo Diels–Alder reaction[15]).

Isolation of **7**[16] and the synthesis of the cis,cis,trans

(5) P. Radlick and S. Winstein, *J. Amer. Chem. Soc.*, **85**, 344 (1963).
(6) K. G. Untch, *ibid.*, **85**, 345 (1963).
(7) K. G. Untch and R. J. Kurland, *ibid.*, **85**, 346 (1963).
(8) K. G. Untch, *J. Mol. Spectrosc.*, **14**, 156 (1964).
(9) This compound was identified by comparison with an authentic sample (mp 105.5–106°, mmp 105–105.5°, superimposable infrared spectra) of azelaic acid.

(10) *trans,trans*-2,4-Hexadiene-1,6-diol was prepared by the method of F. B. Bates, E. R. H. Jones, and M. C. Whiting, *J. Chem. Soc.*, 1854 (1954). Di-*tert*-butyl ester **11** was prepared from the corresponding methyl ester[11] by saponification and esterification of the diacid with isobutylene.[12]
(11) M. J. Goldstein and A. H. Geuirtz, *Tetrahedron Lett.*, 413 (1965).
(12) A. L. McCloskey G. S. Fonken, R. W. Kluiber, and W. S. Johnson, "Organic Syntheses," Collect. Vol. 4, Wiley, New York, N. Y., 1963, p 261.
(13) R. B. Woodward and R. Hoffmann, "The Conservation or Orbital Symmetry," Academic Press, New York, N. Y., 1970.
(14) H. Kwart and K. King, *Chem. Rev.*, **68**, 415 (1968).
(15) J. A. Berson and S. S. Olin, *J. Amer. Chem. Soc.*, **91**, 777 (1969).
(16) Attempts to isolate and purify **7** at low temperatures have been unsuccessful to date. Following the appearance of the low-field olefinic protons of **8** by nmr indicates that the half-life of **7** is *ca.* 15 min at 8°. This is appreciably less than that recently reported for heterocyclic σ-homobenzenes.[17]
(17) H. Prinzbach and D. Stusche, *Angew. Chem.*, **82**, 836 (1970).

isomer **7**, whose $-[{}_{\pi}2_s + {}_{\pi}2_a + {}_{\pi}2_a]$ cycloreversion to **8** is also allowed, is currently under investigation.

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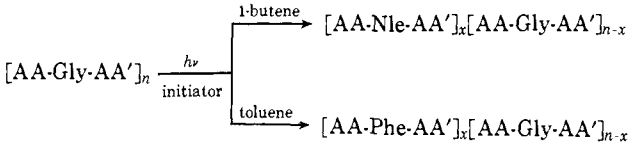
Photoalkylation of Proteins[1]

Sir:

We have recently described the photochemical modification of glycine-containing peptides.[2] This modification consists of the conversion of a glycine derivative into a branched α-amino acid derivative with a preselected side chain. The alkylation process involves substitution of an alkyl or an aralkyl group for an α-hydrogen atom in glycine. Reactions have been applied to the modification of glycine-containing di- and polypeptides, sequential as well as random, and have been found to be selective for glycine residues. The degree of selectivity depends on the location of the glycine residue in the peptide (*i.e.*, on the residue adjacent to the glycine) as well as on the molecular weight and the conformation of the peptide.[2] Examples of such reactions are those with 1-butene or toluene which lead to the conversion of glycine residues into norleucine or phenylalanine, respectively. Initiation of these photoalkylation reactions is achieved by the employment of acetone and ultraviolet light ($\lambda > 260$ nm or > 290 nm) or by a combination of biacetyl + di-*tert*-butyl peroxide (DBP) and visible light.[3] The reactions can be summarized as follows (example given for a sequential copolymer).



We wish to report preliminary results on the application of the photoalkylation reactions to the modification of glycine-containing proteins. The proteins chosen for this study vary greatly in their glycine content: collagen (from tendon; contains 33% glycine), lysozyme (chicken egg white; contains 9.3% glycine, 12 residues of a total of 129), and ribonuclease (bovine pancreatic; contains 2.4% glycine, three residues of a total of 124). In order to avoid ambiguity in the determination of the new α-amino acid produced, side chains not present in the native protein were incorporated into the glycine residues. Thus, norleucine or *p*-fluorophenylalanine, which are produced through the reaction of glycine residues with 1-butene or *p*-fluorotoluene, respectively, were chosen as the new amino acids. The initiation systems were either acetone and ultraviolet light ($\lambda > 260$ nm or > 290 nm), DBP and ultraviolet light ($\lambda > 290$ nm), or biacetyl + DBP and visible light.

In a typical experiment, a mixture of lysozyme (chicken egg white; 20 mg), water (2 ml), *tert*-butyl alcohol (3 ml), and acetone (1.5 ml) was irradiated[4] for 72 hr while 1-butene was bubbled through the mixture. The solvents were removed under reduced pressure and the residue was washed with *tert*-butyl alcohol and dried. The solid was dispersed in water and centrifuged, and the supernatant was fractionated on Sephadex G-75 with 0.1 *M* aqueous acetic acid as eluent. The fractions which exhibited the same mobility as native lysozyme were combined and freeze-dried to yield the modified protein (12 mg) having the amino acid composition[5] presented in Table I.

Table I. Photoalkylation of Lysozyme with 1-Butene Initiated with Acetone and Ultraviolet Light ($\lambda > 290$ nm)

Amino acid	Amino acid composition[a] Native lysozyme	Modified lysozyme
Lysine	5.7 (6)[b]	5.4
Histidine	1.0 (1)	0.2; 0.7[c]
Arginine	10.8 (11)	11
Aspartic acid	21	21
Threonine	7.1 (7)	6.8
Serine	9.9 (10)	9.7
Glutamic acid	5.7 (5)	5.6
Proline	2.3 (2)	2.3
Glycine	12.5 (12)	11.5
Alanine	12.5 (12)	12.3
Half-cystine	6.3 (8)	1.2; 2.0[c]
Valine	5.6 (6)	5.3
Methionine	1.5 (2)	1.5
Isoleucine	5.6 (6)	5.4
Leucine	8.2 (8)	8.1
Tyrosine	3.1 (3)	1.0; 2.3[c]
Phenylalanine	3.0 (3)	
Tryptophan	5.9 (6)[d]	Traces[d]
Norleucine		1.1

[a] Residues/molecule. [b] Figures in parentheses are according to R. E. Canfield, *J. Biol. Chem.*, **238**, 2691 (1963). [c] In the presence of phenol. [d] Determined according to J. R. Spies and D. C. Chambers, *Anal. Chem.*, **21**, 1249 (1949).

The modified lysozyme had no lytic activity on dead cells of *Micrococcus lysodeikticus*[6] and had a slightly lower electrophoretic mobility than native lysozyme on acrylamide gel.[7]

The reactions of the proteins and *p*-fluorotoluene, which led to the conversion of glycine residues into *p*-fluorophenylalanine, were conducted under similar conditions and are summarized in Table II.

Histidine, cystine, methionine, tyrosine, and tryptophan residues were sensitive to the reaction conditions

(1) This work was sponsored by National Institutes of Health, Grant No. AM-10740.
(2) J. Sperling and D. Elad, *J. Amer. Chem. Soc.*, **93**, 967 (1971).
(3) D. Elad, M. Schwarzberg, and J. Sperling, *Chem. Commun.*, 617 (1970).
(4) Hanovia 450-W high-pressure mercury-vapor lamps (Pyrex filters) which were cooled internally with running water were used as the radiation source. Solutions were irradiated 3 cm from the light source in tubes 1.5 cm in diameter.
(5) Amino acid compositions were determined by hydrolysis of the protein in 6 *N* hydrochloric acid at 110° for 22 hr and analysis on a Beckman amino acid analyzer. Values are uncorrected for destruction during hydrolysis.
(6) D. Shugar, *Biochim. Biophys. Acta*, **8**, 302 (1952).
(7) R. A. Reisfeld, U. J. Lewis, and D. E. Williams, *Nature (London)*, **195**, 281 (1962).