Pteridines. XXIX. An Unequivocal Route to 2,4-Diamino-6-substituted Pteridines[1,2]

Edward C. Taylor,* Katherine L. Perlman, Young-Ho Kim, Ian P. Sword, and Peter A. Jacobi

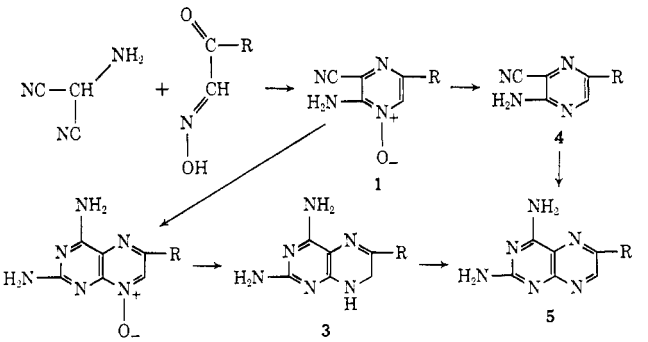
Contribution from the Department of Chemistry, Princeton University, Princeton, New Jersey 08540. *Received February 17, 1973*

Abstract: A versatile new synthetic route to 2,4-diamino-6-substituted pteridines is described. Reaction of an α-ketoaldoxime with aminomalononitrile gives 2-amino-3-cyano-5-substituted pyrazine 1-oxides which yield 2,4-diamino-6-substituted pteridine 8-oxides upon cyclization with guanidine. 2,4-Diaminopteridines are then obtained by deoxygenation of the corresponding 8-oxides, or alternately by prior deoxygenation of the above pyrazine 1-oxides, followed by cyclization with guanidine. The conversion of 2-amino-3-cyano-5-methylpyrazine 1-oxide to the corresponding 1,4-dioxide, and a number of chemical transformations of this latter intermediate, are also described.

In the previous paper in this series,[1] we described a versatile and unequivocal new synthetic route to 6-substituted pterins (2-amino-4(3*H*)-pteridinones). Since 2,4-diaminopteridines serve as precursors to pterins by either alkaline or acid hydrolysis of the 4-amino grouping,[3-5] and are of interest in their own right because of their broad spectrum of biological activities (as dihydrofolate reductase inhibitors,[6] diuretics,[7] antitumor agents,[8] antimalarials,[9] etc.), we turned our attention to the preparation of these compounds by our new procedure. The present paper describes our initial results in this direction.[10]

We have found that the condensation of aminomalononitrile[11] with α-ketoaldoximes leads in high yield to a series of 2-amino-3-cyano-5-substituted pyrazine 1-oxides (**1**). The versatility of *o*-aminonitriles as intermediates for the synthesis of a wide variety of fused 4-aminopyrimidine systems has been exhaustively documented,[12] and one of the most effective of such cyclizations is reaction with guanidine to give fused 2,4-diaminopyrimidines. In the present case, cyclization with guanidine in the presence of sodium methoxide gave a series of 2,4-diamino-6-substituted pteridine 8-oxides (**2**), many of which (like the analogous 6-substituted pterin 8-oxides)[1] were converted into the corresponding 7,8-dihydropteridines (**3**) by sodium dithionite reduction. Subsequent oxidation with potassium permanganate (or even air) then gave 2,4-diamino-6-substituted pteridines (**5**). Alternately, deoxygenation of the pyrazine 1-oxides (**1**) with either phosphorus trichloride or sodium dithionite gave 2-amino-3-cyano-5-substituted pyrazines (**4**) which were cyclized to **5** with guanidine in the presence of sodium methoxide. These reactions are summarized in Scheme I.

Scheme I



a, R = H
b, R = CH_3
c, R = CH_2CH_3
d, R = $CH_2CH_2CH_3$
e, R = $CH(CH_3)_2$
f, R = $CH_2CH(CH_3)_2$
g, R = $(CH_2)_4CH_3$
h, R = $(CH_2)_{16}CH_3$
i, R = CH=NOH
j, R = CH=CHC_6H_5
k, R = C_6H_5
l, R = *o*-$CH_3OC_6H_4$

In one instance, loss of the 3-nitrile grouping accompanied the simple deoxygenation reaction represented by the conversion **1** → **4**. Thus, 2-amino-3-cyano-5-phenylpyrazine 1-oxide (**1k**) upon reduction with sodium dithionite in boiling water gave a mixture of the deoxygenated pyrazine **4k** and 2-amino-5-phenylpyrazine (**7**). The latter compound probably arose by

(1) Part XXVIII: E. C. Taylor, K. L. Perlman, I. P. Sword, M. Sequin-Frey, and P. A. Jacobi, *J. Amer. Chem. Soc.*, **95**, 6407 (1973).

(2) This work was supported by grants to Princeton University from the National Cancer Institute, National Institutes of Health, Public Health Service (Grants No. CA-2551 and 12876) and from the U. S. Army Medical Research and Development Command (Contract No. DA-49-193-2777). This is Contribution No. 1196 in the Army research program on malaria.

(3) D. R. Seeger, D. B. Cosulich, J. M. Smith, Jr., and M. E. Hultquist, *J. Amer. Chem. Soc.*, **71**, 1753 (1949).

(4) E. C. Taylor and C. K. Cain, *ibid.*, **71**, 2538 (1949).

(5) C. M. Baugh and E. Shaw, *J. Org. Chem.*, **29**, 3610 (1964).

(6) R. L. Blakley, "The Biochemistry of Folic Acid and Related Pteridines," Wiley-Interscience, New York, N. Y., 1969.

(7) (a) T. S. Osdene and E. C. Taylor, U. S. Patent 2,975,180 (1961); (b) V. D. Wiebelhaus, J. Weinstock, F. T. Brennan, G. Sosnowski, and T. J. Larsen, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **20**, No. 1, 409 (1961); (c) J. Weinstock and V. D. Wiebelhaus in "Pteridine Chemistry," W. Pfleiderer and E. C. Taylor, Ed., Pergamon Press, Oxford, 1964, pp 37–46; (d) T. S. Osdene, *ibid.*, pp 65–73; (e) J. M. Sprague, *Top. Med. Chem.*, **2**, 49 (1968); (f) J. G. Topliss in "Medicinal Chemistry," 3rd ed, Part II, A. Burger, Ed., Wiley-Interscience, New York, N. Y., 1970, pp 1005–1007.

(8) (a) R. J. Schnitzer and F. Hawking, Ed., "Experimental Chemotherapy," Vol. IV, Part I, Academic Press, New York, N. Y., 1966; (b) L. F. Larionov, "Cancer Chemotherapy," Pergamon Press, Oxford, 1965; (c) F. E. Knock, "Anticancer Agents," Charles C. Thomas, Springfield, Ill., 1966.

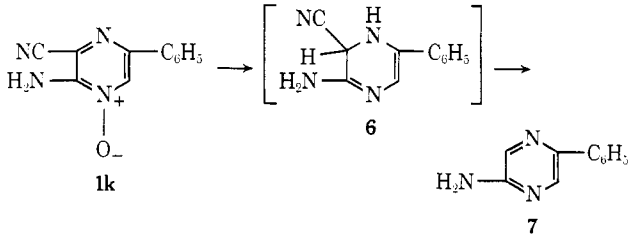
(9) (a) R. M. Pinder in "Medicinal Chemistry," 3rd ed, Part I, A. Burger, Ed., Wiley-Interscience, New York, N. Y., 1970, pp 492–521; (b) J. Hill in "Experimental Chemotherapy," Vol. I, R. J. Schnitzer and F. Hawking, Ed., Academic Press, New York, N. Y., 1966, pp 513–601; (c) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).

(10) A part of this work has appeared in communication form: E. C. Taylor and K. Lenard, *J. Amer. Chem. Soc.*, **90**, 2424 (1968).

(11) (a) J. P. Ferris and L. E. Orgel, *ibid.*, **87**, 4976 (1965); **88**, 3829 (1966); (b) *Org. Syn.*, **48**, 1 (1968).

(12) E. C. Taylor and A. McKillop, "The Chemistry of Cyclic Enaminonitriles and *o*-Aminonitriles," Wiley-Interscience, New York, N. Y., 1970.

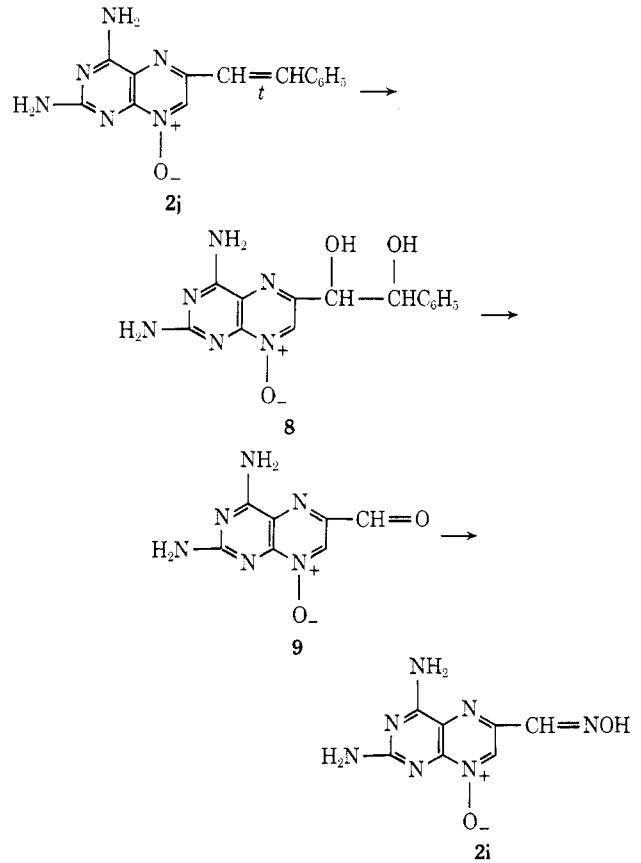
further reduction of the pyrazine ring to the 3,4-dihydro derivative **6** which subsequently aromatized by loss of HCN. Similar side reactions may also have taken place with the other pyrazines (**1**) which were deoxygenated by sodium dithionite, but no effort was made to isolate the small amounts of such by-products which may have been formed.



1k **6** **7**

An attractive potential synthetic route to biopterin and related 1,2-dihydroxyalkyl pterins and pteridines would involve trans hydroxylation of appropriate C-6 trans-olefinic substituents. To this end, some preliminary hydroxylation studies were carried out on 2,4-diamino-6-styrylpteridine 8-oxide (**2j**) (see Scheme II). Reaction with pertrifluoroacetic acid

Scheme II



2j **8** **9** **2i**

(30% hydrogen peroxide in excess trifluoroacetic acid) at room temperature resulted in an apparently rapid reaction, as evidenced by the change in color of the reaction mixture from red to bright yellow over a period of several hours. The product was shown to be the (assumed) *dl-erythro* glycol **8** by spectral data (uv and nmr) and by periodate cleavage to 2,4-diamino-6-formylpteridine 8-oxide (**9**) which was isolated as its oxime **2i**. This latter compound was identical with an authentic sample prepared as described above by cyclization of 2-amino-3-cyano-5-oximinomethylpyrazine 1-oxide (**1i**) with guanidine (see Scheme I).

Preliminary attempts to carry out aldol and Claisen-type reactions on the methyl group of 2-amino-3-cyano-5-methylpyrazine 1-oxide (**1b**) (in an attempt to utilize this readily accessible intermediate for the preparation of olefinic side chains suitable for eventual elaboration into pteridines related to biopterin) were not successful. It was thought that the reactivity of of the C-5 methyl group might be substantially enhanced by conversion of **1b** into the 1,4-dioxide **10**; the enhanced reactivity of the methyl group in 2-picoline 1-oxide *vs.* 2-picoline has been amply demonstrated.[13] Despite the potential plethora of products which could arise by peracid oxidation of **1b** (oxidative removal of the 1-oxide grouping, oxidation of the 2-amino group to a nitro group, conversion of the reactive nitrile grouping to an amide, oxidation of the methyl group, or introduction of oxygen at position 6), we were surprised to find that room temperature oxidation with pertrifluoroacetic acid resulted in smooth conversion of **1b** to 2-amino-3-cyano-5-methylpyrazine 1,4-dioxide (**10**). Its structure was established not only by microanalytical and spectral data (see Experimental Section), but by sodium dithionite reduction in warm aqueous solution to 2-amino-3-cyano-5-methylpyrazine (**4b**), identical with an authentic sample prepared as described above (see Scheme I). Unfortunately, base-catalyzed condensations of **10** with carbonyl compounds were frustrated by the initial conversion of **10** with base into the hydroxamidine anion (the acidity of the 2-amino grouping is apparently substantially increased by di-N-oxidation). Furthermore, strongly nucleophilic bases reacted with **10** with the introduction of a new substituent at position 6. For example, an attempt to condense **10** with guanidine in the presence of sodium methoxide resulted in the formation of 2-amino-3-cyano-5-methyl-6-methoxypyrazine 4-oxide (**11**), which could equally well be prepared from **10** and sodium methoxide in methanol, with omission of guanidine. Attempts to utilize these side reactions for the preparation of 2,4-diamino-7-substituted pteridine 5-oxides were unfortunately unsuccessful; for example, attempted condensation of **11** with guanidine resulted only in displacement of the methoxy grouping to give 2-amino-3-cyano-5-methyl-6-guanidinopyrazine 4-oxide (**12**).

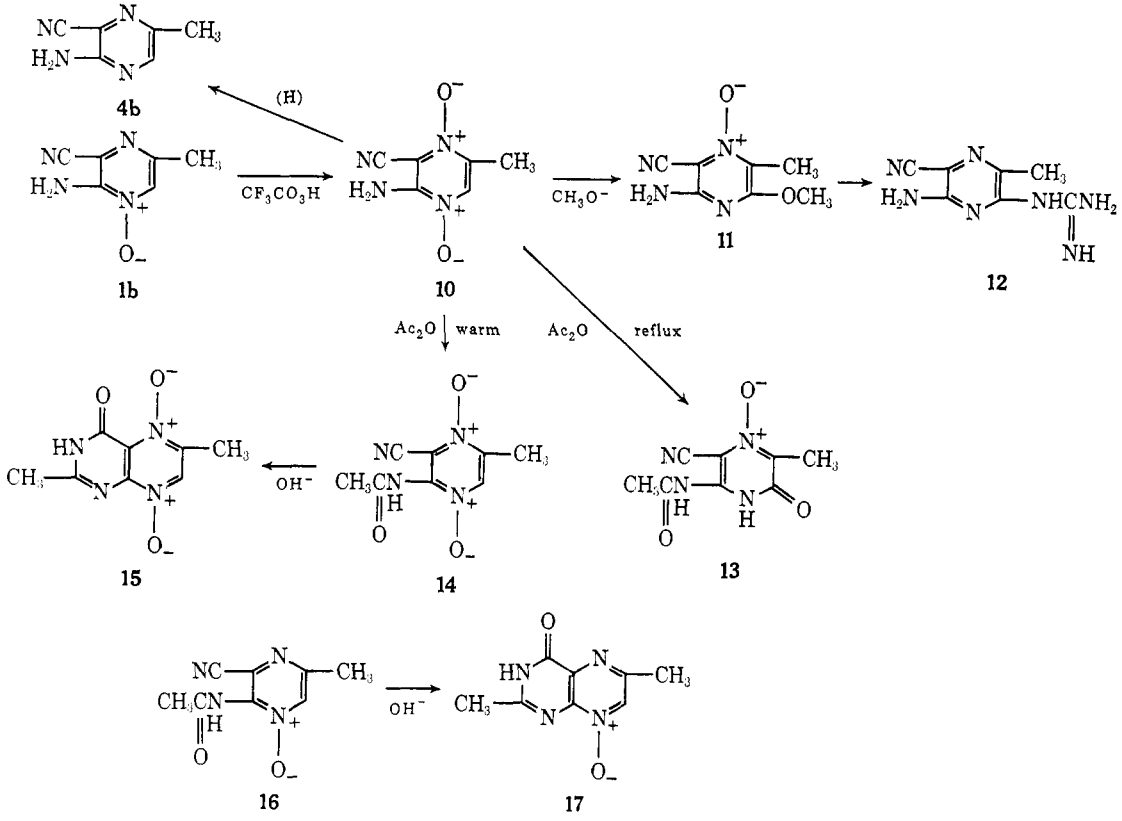
Reaction of **10** with acetic anhydride under reflux led to the formation of the 6(1*H*)-pyrazinone 4-oxide **13**. It is interesting to note that even these vigorous conditions failed to effect acetoxylation of the methyl group, perhaps as a result of decreased basicity of the 4-*N*-oxide grouping. This nitrogen-to-carbon *N*-oxide rearrangement[14] could be avoided by using milder conditions in the reaction of **10** with acetic anhydride. The product of this reaction, which analyzed for a monoacetyl derivative of **10** (*i.e.*, **14**), showed no nitrile absorption in its infrared spectrum (Nujol mull)[15]

(13) A. R. Katritzky and J. M. Lagowski, "Chemistry of the Heterocyclic N-Oxides," Academic Press, New York, N. Y., 1971.

(14) For a recent discussion of this rearrangement, see V. J. Traynelis in "Mechanisms of Molecular Migrations," Vol. 2, B. S. Thyagarajan, Ed., Wiley-Interscience, New York, N. Y., 1969, pp 3–16.

(15) For a discussion of other cyano-substituted heterocycles which fail to exhibit a –CN infrared band, see E. C. Taylor and C. W. Jefford, *J. Amer. Chem. Soc.*, 84, 3744 (1962).

Scheme III



and possessed a high carbonyl frequency band at 1720 cm^{-1}. Similar spectroscopic observations were made on **16**, the product of monoacetylation of **1b**. The presence of nitrile groups in products **14** and **16** was readily confirmed, however, by examination of their Raman spectra, utilizing a He–Ne continuous wave laser operating at 6328.17 Å,[16,17] which clearly revealed nitrile bands at 2247 cm^{-1} in both compounds. Both **14** and **16** were readily reduced with hot aqueous sodium dithionite to 2-acetylamino-3-cyano-5-methylpyrazine and their structures were further confirmed by conversion with hot aqueous alkali[18] into 2,6-dimethyl-4(3*H*)-pteridinone 5,8-dioxide (**15**) and the 8-oxide (**17**), respectively. These reactions are summarized in Scheme III.

The simplicity of this route to 2,4-diaminopteridines, which utilizes readily accessible starting materials, proceeds in high overall yield, and gives products of unequivocal structure, makes it the method of choice both for 2,4-diaminopteridine and (by hydrolysis of the 4-amino grouping) pterin synthesis. Further papers in this series will describe extensions of these reactions to the preparation of biologically significant 2,4-diaminopteridines and of pterin natural products bearing multifunctional C-6 substituents.

Experimental Section[19]

2-Amino-3-cyanopyrazine 1-Oxide (1a). Method A. This compound may be prepared *via* the *in situ* generation and condensation of glyoxal monooxime with aminomalononitrile. To a solution of 40.0 g of 30% aqueous glyoxal and 13.4 g of acetone oxime in 200 ml of water was added 44.0 g of aminomalononitrile tosylate.[11] Complete solution resulted almost immediately, and after about 10 min product started to separate. The reaction mixture was stirred at room temperature for 18 hr and then filtered to give 10.0 g (36%) of golden-brown crystals of 2-amino-3-cyanopyrazine 1-oxide, mp 251–253° dec. Recrystallization from DMF–EtOH gave pale yellow plates: mp 267° dec; nmr (CF_3COOH) δ 8.10 (1, d), 8.28 (1, d).

Deoxygenation with PCl_3 in anhydrous THF, followed by sublimation at 100° (0.1 mm), gave thick yellow needles of 2-amino-3-cyanopyrazine (96% yield), mp 186–188°, identical with an authentic sample.[20]

Method B. A suspension of 17.3 g of powdered glyoxime and 49.5 g of aminomalononitrile tosylate in 160 ml of water was stirred at room temperature for 17 hr. During this time all starting materials passed into solution and a new precipitate formed. The reaction product was collected by filtration and washed with a small amount of cold water and then with ethanol. After drying, the crude product was crystallized from DMF–EtOH to give 7.0 g (26%) of 2-amino-3-cyanopyrazine 1-oxide, identical in all respects with the material prepared by method A above.

2-Amino-3-cyano-5-methylpyrazine 1-Oxide (1b). A suspension of 17.4 g of oximinoacetone and 50.6 g of aminomalononitrile tosylate in 300 ml of 2-propanol was stirred at room temperature for 4 hr. The product crystallized directly from the reaction mixture as yellow crystals which were collected by filtration, washed well with a small amount of cold water followed by cold ethanol, and dried *in vacuo;* yield 25.0 g (83%), mp 187–188°. Recrystallization from ethanol did not change the melting point: nmr (CD_3COOD) δ 2.00 (3, s, C_5-CH_3), 8.03 (1, s, C_6-H); uv $\lambda_{max}^{C_2H_5OH}$ nm (log ϵ) 224 (4.08), 252 (4.29), 285 sh (3.63), 380 (3.81).

(16) R. E. Miller, D. L. Rousseau, and G. E. Leroi, Technical Report No. 22, ONR Contract 1858(27), NRO14-203, May 1967 (available from the Defense Documentation Center, Cameron Station, Alexandria, Va. 22314).

(17) We are indebted to Professor T. G. Spiro of this department for assistance with this experiment.

(18) See ref 12, pp 226–231.

(19) Melting points are uncorrected and were determined on a Thomas-Hoover apparatus. Infrared data refer to Nujol mull spectra taken on a Perkin-Elmer 237B grating infrared spectrometer. Nmr data were obtained on a Varian A-60A instrument, using TMS as internal standard. Elemental analyses for all new compounds were in agreement with the assigned structures to within 0.4% except for 2-acetylamino-3-cyano-5-methylpyrazine 1-oxide (0.5%) and **5h** (0.6%). Results of these analyses were made available to the editor.

(20) (a) R. C. Ellingson, R. L. Henry, and F. G. McDonald, *J. Amer. Chem. Soc.*, **67**, 1711 (1945); (b) E. C. Taylor, R. J. Knopf, J. A. Cogliano, J. W. Barton, and W. Pfleiderer, *ibid.*, **82**, 6058 (1960).

Heating the above compound for a few minutes in excess acetic anhydride, followed by dilution with ethanol, evaporation, and crystallization of the residue from ethanol, gave 2-acetylamino-3-cyano-5-methylpyrazine 1-oxide as colorless rods, mp 174°.

2-Amino-3-cyano-5-ethylpyrazine 1-Oxide (1c). This compound was prepared in 81% yield as pale yellow needles, mp 139–140° dec, by stirring a mixture of aminomalononitrile tosylate (12.1 g) and 1-oximino-2-butanone (4.6 g) in 50 ml of 2-propanol overnight, filtering, and recrystallizing the crude product (6.0 g) from ethanol: nmr (CF_3COOH) δ 0.86 (3, t), 2.43 (2, q, CH_2CH_3), 8.00 (1, s, C_6-H).

2-Amino-3-cyano-5-*n*-propylpyrazine 1-Oxide (1d). This compound (13.0 g) was prepared in 84% yield as yellow needles, mp 145–146° dec, by stirring a mixture of 22.0 g of aminomalononitrile tosylate and 10.0 g of 1-oximino-2-pentanone in 2-propanol overnight, filtering, and recrystallizing the crude product from ethanol: nmr ($CDCl_3$) δ 0.92 (3, t, J = 9 Hz), 1.70 (2, m), 2.62 (2, t, J = 9 Hz, C_3H_7), 8.20 (1, s, C_6-H); 6.40 (2, br, NH_2); uv $\lambda_{max}^{CH_3OH}$ nm (log ϵ) 229 (3.83), 252 (4.05), 378 (3.46).

2-Amino-3-cyano-5-isopropylpyrazine 1-Oxide (1e). This compound was prepared in 71% yield as pale yellow needles, mp 126–128° dec (from ethanol), by condensation of aminomalononitrile tosylate and 1-oximino-3-methyl-2-butanone in 2-propanol, as described above: nmr (CF_3COOH) δ 0.84 (6, d), 2.65 (1, hep, $CH(CH_3)_2$), 7.93 (1, s, C_6-H).

2-Amino-3-cyano-5-isobutylpyrazine 1-Oxide (1f). This compound was prepared in 77% yield as pale yellow needles, mp 140–142° dec (from ethanol), by condensation of aminomalononitrile tosylate and 1-oximino-4-methyl-2-pentanone in 2-propanol, as described above: nmr (CF_3COOH) δ 0.48 (6, d), 1.60 (1, m), 2.25 (2, d, isobutyl), 7.95 (1, s, C_6-H).

2-Amino-3-cyano-5-*n*-pentylpyrazine 1-Oxide (1g). This compound was prepared in 63% yield as pale yellow needles, mp 103–104° dec (from ethanol), by condensation of aminomalononitrile tosylate and 1-oximino-2-heptanone in 2-propanol, as described above: nmr (CF_3COOH) 0.42 (3, t), 1.15 (6, m), 2.38 (2, t, *n*-pentyl), 7.98 (1, s, C_6-H).

2-Amino-3-cyano-5-heptadecylpyrazine 1-Oxide (1h). Ethyl stearoyl acetate was converted to 3-oxodocosanoic acid by the method of Mitz, *et al.*,[21] using the modification described by Stenhagen.[22] A suspension of 3.3 g of this β-keto acid in 75 ml of dry ether was saturated with dry hydrogen chloride at 0°, and 1.3 g of isoamyl nitrite was added dropwise, with stirring. After 2 hr at room temperature, the resulting almost colorless solution was washed well with ice–water, dried over Na_2SO_4, and evaporated at room temperature in a stream of air to give 3.2 g of a waxy solid, mp 65–66° (with gas evolution). This material was heated on a steam bath until gas evolution ceased (~3 min), and the resulting light yellow wax was dissolved in ether–pentane and passed through a column of silica gel. Evaporation of the eluate then gave 2.2 g of oximinomethyl heptadecyl ketone as a colorless wax which was condensed directly with aminomalononitrile tosylate (in 2-propanol–methanol), as described above in the preparation of **1a**. 2-Amino-3-cyano-5-heptadecylpyrazine 1-oxide was formed in 68% yield as yellow crystals, mp 105–108° (from methanol).

2-Amino-3-cyano-5-oximinomethylpyrazine 1-Oxide (1i). This compound was prepared in quantitative yield as pale yellow needles, mp 300° dec, from aminomalononitrile tosylate and dioximinoacetone in 2-propanol, as described above. The analytical sample was recrystallized from DMF: nmr (DMSO-d_6) δ 8.32 (1, s, CH=NOH), 7.68 (1, s, C_6-H), 7.9 (2, br, NH_2); uv $\lambda_{max}^{0.1\,N\,NaOH}$ nm (log ϵ) 324 sh (4.24); $\lambda_{max}^{CH_3OH}$ nm (log ϵ) 276 sh (4.33), 390 (3.72).

2-Amino-3-cyano-5-styrylpyrazine 1-Oxide (1j). This compound was prepared in 70% yield as bright yellow needles, mp 206–207° dec (from ethanol), by condensation of aminomalononitrile tosylate with styryl oximinoketone in 2-propanol: nmr (DMSO-d_6) δ 8.74 (1, s, C_6-H), 7.90 (2, s, NH_2), 7.3 (7, br m, C_6H_5CH=CH); uv $\lambda_{max}^{CH_3OH}$ nm (log ϵ) 297 (4.40), 313 (4.43), 383 (4.10).

2-Amino-3-cyano-5-phenylpyrazine 1-Oxide (1k). This compound was prepared in 78% yield as pale yellow needles, mp 214° (from ethanol), by condensation of aminomalononitrile tosylate with oximinoacetophenone in 2-propanol: nmr (DMSO-d_6) δ 8.70 (1, s, C_6-H), 6.5 (7, br m, C_6H_5, NH_2); $\lambda_{max}^{CH_3OH}$ nm (log ϵ) 281 (sh) (4.41), 391 (3.78).

2-Amino-3-cyano-5-(*o*-methoxyphenyl)pyrazine 1-Oxide (1l). This compound was prepared in 66% yield as pale yellow needles, mp 251–253° dec (from ethanol), by condensation of aminomalononitrile tosylate and *o*-methoxybenzoylformaldoxime in 2-propanol, as described above: nmr (CF_3COOH) δ 3.50 (3, s, OCH_3), 7.10 (4, m, C_6H_4), 8.86 (1, s, C_6-H).

2-Amino-3-cyano-5-heptadecylpyrazine (4h). The general procedure for reduction of pyrazine *N*-oxides to parent pyrazines was to treat a solution of the *N*-oxide in THF with phosphorus trichloride, concentrate the reaction mixture, and pour over ice. The desired product was then obtained either by direct filtration or by extraction with an appropriate organic solvent. Thus, a solution of 5.5 g of 2-amino-3-cyano-5-heptadecylpyrazine 1-oxide in 200 ml of dry THF maintained at 0° was treated slowly, over a period of 5 min, with 30 ml of phosphorus trichloride. The reaction mixture was stirred at room temperature for 40 min, concentrated to *ca.* 50 ml by evaporation under reduced pressure, and poured into 1 l. of ice–water. The product which precipitated was collected by filtration and recrystallized from methanol; yield 5.0 g (90%), mp 128–130°.

The following compounds were prepared in the same manner in 75–95% yield by reduction of the corresponding 1-oxides with PCl_3. Somewhat less satisfactory yields (50–70%) were obtained by reduction with aqueous sodium dithionite.

2-Amino-3-cyano-5-methylpyrazine (4b): mp (purified by sublimation) 172–173°; nmr (DMSO-d_6) δ 2.12 (3), s, C_5-CH_3), 6.64 (2, br, NH_2), 7.83 (1, s, C_6-H); uv $\lambda_{max}^{CH_3OH}$ nm (log ϵ) 249 (4.17), 358 (3.88).

2-Amino-3-cyano-5-*n*-propylpyrazine (4d): mp (from ethanol) 138°; nmr (CF_3COOH) 0.57 (3, t, J = 8 Hz), 1.39 (2, m), 2.45 (2, t, J = 8 Hz, C_3H_7), 7.65 (1, s, C_6-H).

2-Amino-3-cyano-5-styrylpyrazine (4j): mp (from CH_3COOH) 192°; nmr (CF_3COOH) δ 7.50 (1, s, C_6-H), 6.20–6.30 (7, m, C_6H_5CH=CH).

2-Amino-3-cyano-5-phenylpyrazine (4k) had mp (from ethanol) 182°.

Extraction with ether of the mother liquors of the aqueous dithionite reduction mixture, followed by drying (Na_2SO_4) and evaporation, gave *2-amino-5-phenylpyrazine* (**7**) as pale yellow needles: mp (from water) 140–141°; nmr (CF_3COOH) δ 6.40 (1, s, C_6-H); 7.2 (5, m, C_6H_5), 7.87 (1, d, J = ~2 Hz, C_3-H).

Acetylation of this latter compound with acetic anhydride gave 2-acetylamino-5-phenylpyrazine, mp (from petroleum ether, bp 60–70°) 161–162°.

2,4-Diaminopteridine 8-Oxide (2a). Guanidine hydrochloride (2.4 g) was added to a solution of 1.5 g of sodium in 200 ml of absolute methanol, and the precipitated sodium chloride was removed by filtration. To the filtrate was added 2.9 g of 2-amino-3-cyanopyrazine 1-oxide, and the resulting mixture was heated under reflux with stirring for 16 hr. A yellow precipitate started to separate from the orange solution after approximately 1 hr. The mixture was cooled and filtered and the collected solid washed well with methanol and dried to give 3.4 g (90%) of 2,4-diaminopteridine 8-oxide, mp >300°. The analytical sample was prepared by recrystallization from a large volume of DMF: nmr (CF_3COOH) δ 8.21 (1, d), 8.46 (1, d); uv $\lambda_{max}^{0.1\,N\,HCl}$ nm (log ϵ) 250 (4.42), 280 (3.93), 353 (3.88), 367 (3.84).

The following compounds were prepared in the same manner from guanidine and the corresponding 2-amino-3-cyano-5-substituted pyrazine 1-oxides.

2,4-Diamino-6-methylpteridine 8-Oxide (2b): 81% yield, mp >320° (purified by dissolution in dilute HCl followed by precipitation with NH_4OH); nmr (CF_3COOH) δ 2.25 (3, s, C_6-CH_3), 8.43 (1, s, C_7-H); uv $\lambda_{max}^{H_2O}$ nm (log ϵ) 213 (4.12), 228 sh (3.89), 263 (4.48), 293 sh (3.84), 392 (3.84).

2,4-Diamino-6-ethylpteridine 8-Oxide (2c): 70% yield, mp 301° dec (from DMF); nmr (CF_3COOH) δ 0.96 (3, t), 2.65 (2, q, CH_2CH_3), 8.49 (1, s, C_7-H); uv $\lambda_{max}^{0.1\,N\,HCl}$ nm (log ϵ) 251 (4.46), 281 (3.96), 357 (3.91), 371 (3.88).

2,4-Diamino-6-*n*-propylpteridine 8-Oxide (2d): 86% yield, mp (from DMF) 290–292°; nmr (CF_3COOH) δ 0.60 (3, t), 1.44 (2, q), 2.56 (2, t, C_3H_7), 8.29 (1, s, C_7-H); uv $\lambda_{max}^{0.12\,N\,HCl}$ nm (log ϵ) 251 (4.40), 282 (3.89), 369 (3.79).

2,4-Diamino-6-isopropylpteridine 8-Oxide (2e): 67% yield, mp 285° dec (from DMF); nmr (CF_3COOH) δ 1.00 (6, d), 2.90 (1, hep, $CH(CH_3)_2$), 8.36 (1, s, C_7-H); uv $\lambda_{max}^{0.1\,N\,HCl}$ nm (log ϵ) 251 (4.44), 282 (3.92), 357 (3.89), 370 (3.84).

2,4-Diamino-6-isobutylpteridine 8-Oxide (2f): 83% yield, mp 279° dec (from DMF); nmr (CF_3COOH) δ 0.56 (6, d), 1.79 (1, m), 2.45 (2, d, isobutyl), 8.30 (1, s, C_7-H); uv $\lambda_{max}^{0.1\,N\,HCl}$ nm (log ϵ) 2.52 (4.48), 282 (3.98), 357 (3.91), 372 (3.88).

(21) M. A. Mitz, A. E. Axelrod, and K. Hofmann, *ibid.*, 72, 1231 (1950).

(22) E. Stenhagen, *Ark. Kemi*, 3, 381 (1951).

2,4-Diamino-6-*n*-pentylpteridine 8-Oxide (2g): 74% yield, mp 258° dec (from DMF); nmr (CF_3COOH) δ 0.45 (3, t), 1.12 (6, m), 2.55 (2, t, *n*-pentyl), 8.27 (1, s, C_7-H); uv $\lambda_{max}^{0.1\,N\,HCl}$ nm (log ϵ) 250 (4.37), 283 (3.85), 357 (3.79), 372 (3.76).

2,4-Diamino-6-heptadecylpteridine 8-Oxide (2h): 81% yield, mp 244–245° (after leaching with boiling methanol and water).

2,4-Diamino-6-oximinomethylpteridine 8-Oxide (2i): 89% yield, mp >350° (because of the extreme insolubility of this compound, impurities were leached from the crude product by boiling first with DMF and then with water); uv $\lambda_{max}^{0.1\,N\,NaOH}$ nm (log ϵ) 275 (4.29), 313 (4.41).

2,4-Diamino-6-styrylpteridine 8-Oxide (2j): 97% yield, mp (from DMF) 275° dec; nmr (CF_3COOH) δ 6.70 (1, d), 7.40 (1, d, J = 16 Hz, CH=CH), 7.0 (5, m, C_6H_5), 8.40 (1, s, C_7-H); uv $\lambda_{max}^{0.12\,N\,HCl}$ nm (log ϵ) 280 (4.19), 323 (4.49).

2,4-Diamino-6-phenylpteridine 8-Oxide (2k): 100% yield, mp >300° (from DMF); uv $\lambda_{max}^{0.12\,N\,HCl}$ nm (log ϵ) 275 (4.49), 375 (3.92).

2,4-Diamino-6-*o*-methoxyphenylpteridine 8-Oxide (21): 100% yield, mp 290° dec (from DMF); nmr (CF_3COOH) δ 3.53 (3, OCH_3), 7.18 (4, m, C_6H_4), 9.21 (1, s, C_7-H); uv $\lambda_{max}^{0.1\,N\,HCl}$ nm (log ϵ) 259 (4.30), 276 sh (4.26), 294 (4.14), 383 (3.81).

2,4-Diamino-6-methyl-7,8-dihydropteridine (3b). A suspension of 1.5 g of 2,4-diamino-6-methylpteridine 8-oxide (**2b**) in 30 ml of boiling water was treated portionwise with sodium dithionite until the bulk of the suspended solid had passed into solution. The hot aqueous solution was filtered to remove 0.15 g of suspended solid; cooling of the filtrate gave 1.3 g (65%) or colorless crystals of 2,4-diamino-6-methyl-7,8-dihydropteridine hydrosulfite, mp >280° dec (from water).

The free base was obtained in quantitative yield and in a high state of purity by stirring an aqueous suspension of the sulfurous acid salt above with dilute sodium hydroxide solution to give colorless crystals: mp >300° dec (from water); nmr (CF_3COOH) δ 3.08 (3, s, C_6-CH_3), 4.40 (2, s, C_7-CH_2); uv $\lambda_{max}^{0.1\,N\,HCl}$ nm (log ϵ) 232 (4.28), 288 (4.06).

2,4-Diamino-6-methylpteridine (5b). Method A. A suspension of 2,4-diamino-6-methyl-7,8-dihydropteridine in hot water was treated with dilute potassium permanganate solution to give 2,4-diamino-6-methylpteridine[3], mp >340°, in quantitative yield: nmr (CF_3COOH) δ 2.30 (3, s, C_6-CH_3), 8.16 (1, s, C_7-H); uv $\lambda_{max}^{0.12\,N\,HC}$ nm (log ϵ) 242 (4.16), 281 (3.63), 338 (3.99), 349 (3.93).

Method B. To a solution of 2.8 g of sodium methoxide in 100 ml of anhydrous methanol was added 1.9 g of guanidine hydrochloride and the resulting supension filtered from sodium chloride. The filtrate was then added to 2.1 g of 2-amino-3-cyano-5-methylpyrazine and the reaction mixture refluxed overnight. Cooling and filtering gave a light yellow solid which was washed well with water and dried; yield 2.3 g (84%). The product was identical in all respects with the compound prepared by method A above.

2,4-Diamino-6-*n*-propyl-7,8-dihydropteridine (3d). Reduction of 2,4-diamino-6-*n*-propylpteridine 8-oxide (**2d**) with sodium dithionite, as described above for the conversion of **2b** to **3b**, gave 2,4-diamino-6-*n*-propyl-7,8-dihydropteridine hydrosulfite monohydrate, mp 250° dec (from water).

Ammonium hydroxide liberated the free base, which upon recrystallization from water was immediately oxidized to 2,4-diamino-6-*n*-propylpteridine, mp 282°, identical with an authentic sample (see below). The nmr spectrum (CF_3COOH) of the crude dihydro compound, however, showed the C_7–CH_2 signals at δ 4.5.

2,4-Diamino-6-*n*-propylpteridine (5d). This compound was prepared in 80% yield by condensation of 2-amino-3-cyano-5-*n*-propylpyrazine with guanidine, as described above for the preparation of **5b**: mp (from dimethylacetamide) 282°; nmr (CF_3COOH) δ 0.62 (3, t, J = 7 Hz), 1.50 (2, m), 2.67 (2, t, J = 7 Hz, C_3H_7), 8.40 (1, s, C_7-H); uv $\lambda_{max}^{0.1\,N\,HCl}$ nm (log ϵ) 243 (4.22), 278 (3.75), 338 (4.02), 351 (3.97).

2,4-Diamino-6-heptadecylpteridine (5h). This compound was prepared in 95% yield by condensation of 2-amino-3-cyano-5-heptadecylpyrazine with guanidine, mp (from aqueous DMF) 249–251° dec.

2,4-Diamino-6-styrylpteridine (5j). This compound was prepared in 95% yield by condensation of 2-amino-3-cyano-5-styrylpyrazine with guanidine: mp (from dimethylacetamide–petroleum ether, bp 60–70°) 342° dec; nmr (CF_3COOH) δ 7 (7, br m, CH=CHC_6H_5), 8.46 (1, s, C_7-H); uv $\lambda_{max}^{0.1\,N\,HCl}$ nm (log ϵ) 244 (4.06), 306 sh (4.38), 386 (4.10).

2,4-Diamino-6-phenylpteridine (5k). This compound was prepared in 85% yield by condensation of 2-amino-3-cyano-5-phenylpyrazine with guanidine: mp (from DMF) 352° dec (lit.[23] mp 340°dec); nmr (CF_3COOH) δ 7.5 (5, m, C_6H_5), 8.94 (1, s, C_7-H); uv $\lambda_{max}^{0.1\,N\,HCl}$ nm (log ϵ) 265 (4.80), 363 (4.17).

2,4-Diamino-6-(*d,l-erythro*-1,2-dihydroxy-2-phenylethyl)pteridine 8-Oxide (8). A solution of 2.1 g of 2,4-diamino-6-styrylpteridine 8-oxide in 10 ml of trifluoroacetic acid containing 2 ml of 30% hydrogen peroxide was stirred at room temperature for 2 hr and poured into ice, and the resulting precipitate was collected by filtration. It was then stirred in suspension with excess 2 *N* sodium hydroxide solution for 30 min at room temperature, washed well with water, and recrystallized from water to give 1.2 g (51%): mp 243° dec; nmr (CF_3COOH) δ 4.05 (2, q, CH-CH), 6.05 (5, s, C_6H_5), 7.40 (1, s, C_7-H); uv $\lambda_{max}^{0.1\,N\,HCl}$ nm (log ϵ) 255 (4.49), 280 (4.06), 357 (390), 370 (3.84).

A suspension of 0.20 g of **8** in 4 ml of water containing 0.175 g of hydrated periodic acid was stirred overnight at room temperature and then filtered. The collected solid was suspended in 1 ml of pyridine containing 0.10 g of hydroxylamine hydrochloride, stirred overnight, and then poured into water. After standing at room temperature for several hours, the mixture was filtered to give a yellow granular solid which was washed well with water and dried. It was identical in all respects with an authentic sample of 2,4-diamino-6-oximinomethylpteridine 8-oxide (**2i**).

The aqueous reaction filtrate above was extracted with 2 × 10-ml portions of ether; the extracts were dried (Na_2SO_4) and evaporated to give benzaldehyde, identified by comparison of glc retention times with an authentic sample.

2-Amino-3-cyano-5-methylpyrazine 1,4-Dioxide (10). To a well-chilled solution of 11.4 ml of 30% hydrogen peroxide in 100 ml of trifluoroacetic anhydride was added 10.0 g of 2-amino-3-cyano-5-methylpyrazine 1-oxide, and the mixture was stirred at room temperature for 40 hr. It was then concentrated *in vacuo* to about 30 ml and diluted with petroleum ether (bp 30–60°). The yellow solid which separated was collected by filtration and recrystallized from formamide to give 5.7 g (51%) of bright yellow needles: mp 221° dec; nmr (DMSO-d_6) δ 2.17 (3, s, C_5-CH_3), 8.61 (1, s, C_6-H), 7.96 (2, br s, NH_2); uv $\lambda_{max}^{CH_3OH}$ nm (log ϵ) 238 (4.37), 256 (4.16), 310 (3.91).

The mother liquors from the above oxidation reaction were evaporated to dryness in a stream of air, the tarry residue was dissolved in 30 ml of aqueous ethanol, and 2.0 g of sodium dithionite was added in small portions. The cooled reaction mixture was then extracted with chloroform; the extracts were dried (Na_2SO_4) and evaporated to give 0.8 g of colorless 2-amino-3-cyano-5-methylpyrazine (**4b**), identical with an authentic sample.

2-Acetylamino-3-cyano-5-methylpyrazine 1,4-Dioxide (14). Treatment of 2-amino-3-cyano-5-methylpyrazine 1,4-dioxide with warm acetic anhydride resulted in rapid separation from the reaction mixture of golden yellow rods, mp 203° dec (from acetic acid), in quantitative yield: nmr (CF_3COOH) δ 2.26 (3, s, C_5-CH_3), 2.60 (3, s, CH_3C=O), 9.0 (1, s, C_6-H); ir 3100, 1720 cm^{-1}; Laser Raman 2247 cm^{-1} (CN).

Addition of sodium dithionite to a hot aqueous suspension of this compound gave, on cooling, colorless bipyramidal crystals, mp 182° (from ethanol), of 2-acetylamino-3-cyano-5-methylpyrazine.

2-Acetylamino-3-cyano-5-methyl-6(1*H*)-pyrazinone 4-Oxide (13). A solution of 0.50 g of 2-amino-3-cyano-5-methylpyrazine 1,4-dioxide in 3 ml of acetic anhydride was heated under reflux for several minutes. Dilution of the black reaction mixture with ethanol and evaporation gave a residual dark solid which was crystallized from ethanol to give 0.15 g (24%) of colorless crystals: mp 245° dec; nmr (DMSO-d_6) δ 2.03 (3, s), 2.06 (3, s, C_5-CH_3, CH_3C=O); ir 3120, 2230, 1690, 1630 cm^{-1}.

2,6-Dimethyl-4(3*H*)-pteridinone 5,8-Dioxide (15). A solution of 0.50 g of 2-acetylamino-3-cyano-5-methylpyrazine 1,4-dioxide in 3 ml of 2.5 *N* sodium hydroxide was heated for 1 min at 100° and then cooled. The insoluble sodium salt of the product which separated was collected by filtration, dissolved in hot water, and passed through a column containing 5 g of Amberlite IR120 cation exchange resin. Evaporation of the eluates gave 0.25 g (50%) of **15** as colorless crystals, mp >300°.

2,6-Dimethyl-4(3*H*)-pteridinone 8-Oxide (17). A solution of 0.1 g of 2-acetylamino-3-cyano-5-methylpyrazine 1-oxide in 1 ml of 2.5 *N* sodium hydroxide was heated for 1 min at 100° and cooled, and the bright yellow sodium salt of **17** was collected by filtration. Dissolution in hot water followed by acidification with acetic acid resulted in the separation of 0.8 g (80%) of colorless needles, mp >300°.

(23) I. J. Pachter and P. E. Nemeth, *J. Org. Chem.*, **28**, 1203 (1963).

2-Amino-3-cyano-5-methyl-6-methoxypyrazine 4-Oxide (11). A solution of 0.50 g of 2-amino-3-cyano-5-methylpyrazine 1,4-dioxide in 20 ml of dry methanol containing 0.43 g of sodium methoxide was heated under reflux overnight. The resulting brownish-red solution was evaporated to about half its volume under reduced pressure, cooled, and filtered. The collected solid was crystallized from acetic acid (charcoal) to give 0.25 g (46%) of colorless crystals: mp >235° dec; nmr (CF_3COOH) 2.07 (3, s, C_5-CH_3), 3.71 (3, s, OCH_3).

2-Amino-3-cyano-5-methyl-6-guanidinopyrazine 4-Oxide (12). A mixture of 0.20 g of 2-amino-3-cyano-5-methyl-6-methoxypyrazine 4-oxide, 0.32 g of guanidine hydrochloride, and 0.23 g of sodium methoxide in 12 ml of dry methanol was heated under reflux for 14 hr. The yellow solid which had separated was collected by filtration and crystallized from glacial acetic acid. The resulting acetate salt of **12** was stirred with aqueous sodium bicarbonate to give a colorless solid which was crystallized from aqueous DMF: yield 0.13 g (57%); mp >280° dec; ir 2210 cm^{-1} (CN).

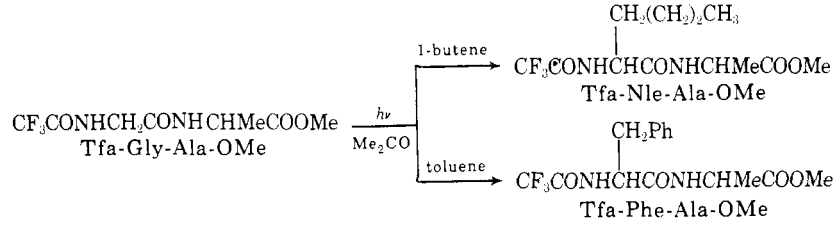
Photoalkylation of Peptides. Visible Light-Induced Conversion of Glycine Residues into Branched α-Amino Acids

M. Schwarzberg,[1] J. Sperling, and D. Elad*

Contribution from the Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel. *Received April 14, 1973*

Abstract: Glycine residues in dipeptides and polypeptides have been modified by a photoalkylation procedure which results in their preferential conversion into branched α-amino acids. The reactions were induced with visible light using a combination of an α-diketone and a peroxide as the photoinitiator. The degree of selectivity and that of the asymmetric induction were found to increase with the increase in molecular weight of the peptide. Results indicate that the reactions proceed through energy transfer from the photoexcited triplet α-diketone to the peroxide leading subsequently to fragmentation of the latter and thereby to a free-radical reaction.

Photochemical modifications of glycine-containing peptides and proteins have been described by us in a series of publications.[2] These modifications involve an alkylation process by which glycine residues are converted into residues of a variety of branched α-amino acids through the substitution of a preselected alkyl or aralkyl group for a hydrogen atom at the α position of the glycine. The reactions were induced photochemically with ultraviolet light (λ >260 nm or >290 nm) using acetone as a photoinitiator, and were found to be selective for glycine residues in peptides and proteins. While employing 1-butene or toluene as reagents, glycine residues were converted into norleucine and phenylalanine, respectively. A mechanism



involving free-radical intermediates has been proposed for these reactions.[2a] In the initiation step an excited acetone molecule abstracts a hydrogen atom from the α carbon of glycine, thus leading to a free radical which subsequently reacts with an olefin (*e.g.*, 1-butene) or an aralkyl radical (*e.g.*, benzyl) to yield the new branched α-amino acid.

The extension of these photoalkylation reactions to peptides and proteins containing ultraviolet-sensitive amino acids, as well as the incorporation of sensitive amino acid residues (*e.g.*, tyrosine) into a protein molecule, may require the use of light of longer wavelengths and employment of suitable photoinitiators. The use of a variety of photoinitiators might also lead to a broader scope of these modification reactions and to the possible incorporation of new side chains into glycine (*e.g.*, the production of aspartic acid or serine derived from acetic acid[3] or methanol,[4] respectively). α-Diketones could be considered as suitable photoinitiators for these reactions, since they absorb ultraviolet light of long wavelengths, and their absorption extends into the visible region. However, the hydrogen atom abstraction ability of diketones in the excited state is rather weak as compared to that of the corresponding monoketones.[5] Indeed, we found that α-diketones failed to initiate the photoalkylation reactions. Peroxides, such as di-*tert*-butyl peroxide (DBP), afford free radicals which are powerful hydrogen atom abstracting agents and may be considered

(1) In partial fulfillment of the requirements for a Ph.D. Thesis submitted to the Feinberg Graduate School, 1972.

(2) (a) D. Elad and J. Sperling, *J. Chem. Soc. C*, 1579 (1969); (b) J. Sperling and D. Elad, *J. Amer. Chem. Soc.*, **93**, 967 (1971); (c) *ibid.*, **93**, 3839 (1971), and references cited therein.

(3) J. C. Allen, J. I. G. Cadogan, and D. H. Hey. *J. Chem. Soc.*, 1918 (1965).

(4) W. H. Urry, F. W. Stacey, E. S. Huyser, and O. O. Juveland, *J. Amer. Chem. Soc.*, **76**, 450 (1954).

(5) O. W. Jolly and P. de Mayo, *Can. J. Chem.*, **42**, 170 (1964); W. G. Bentrude and K. R. Darnall, *Chem. Commun.*, 810 (1968); M. B. Rubin, *Fortschr. Chem. Forsch.*, **13**, 251 (1969), and references cited therein; N. J. Turro and T. J. Lee, *J. Amer. Chem. Soc.*, **91**, 5651 (1969); B. Monroe, *Advan. Photochem.*, **8**, 77 (1971).