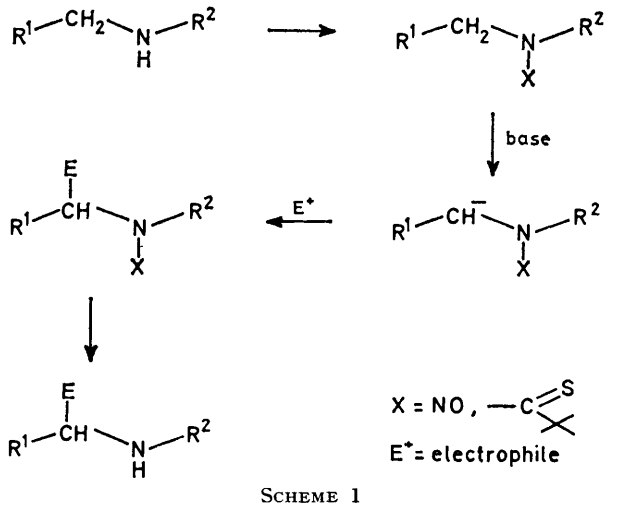
Reactions of Relevance to the Chemistry of Aminoglycoside Antibiotics. Part 10.† α-Alkylation of Primary Amines

By **Derek H. R. Barton,* Geneviève Lamotte, William B. Motherwell,** and **Subhash C. Narang,** Chemistry Department, Imperial College, London SW7 2AY, and Institut de Chimie des Substances Naturelles, C.N.R.S., 91190 Gif-sur-Yvette, France

Alkylation at the α-carbon atom of primary amines can be conveniently accomplished by a sequence involving formation of the corresponding azoxy-derivative, alkylation with an alkyl-lithium, and subsequent cleavage of the α-alkylated azo-compound. Improved procedures are described for the preparation of unsymmetrical azoxy-derivatives. The scope of the reductive alkylation of azoxy-compounds has been considerably extended.

CHAIN extension by alkylation at the α-carbon atom of amines is a desirable reaction, particularly for the modification of biological activity in aminoglycoside antibiotics. Intensive research in this area has thus far uncovered two successful methods,[1-3] both of which derive from activation of the α-carbon atom of a secondary amine towards electrophilic alkylation by substitution at the nitrogen atom with an electron-withdrawing group capable of stabilising an adjacent carbanion. This general approach is set out in Scheme 1.

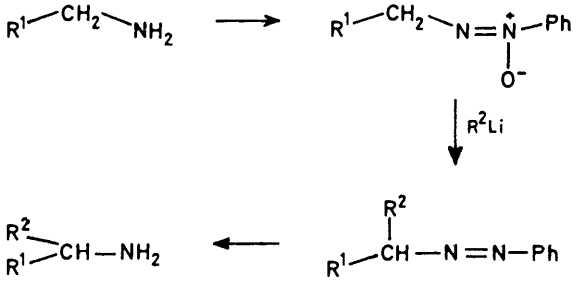
We sought, however, to develop a process which would be applicable to primary amines. The literature suggests



SCHEME 1

that conversion of an amine into the corresponding electron-withdrawing azoxy-group does not facilitate ionisation of the adjacent C–H bond (pK_as >14).[4] Nevertheless, we were intrigued by an isolated report [5] that reaction of an aliphatic azoxy-compound with an alkyl-lithium gave an α-alkylated azo-compound, albeit in low yield. Since subsequent reduction of the azo-moiety to an amine is well known, the sequence in Scheme 2 was proposed for chain extension at the α-carbon atom of primary amines.

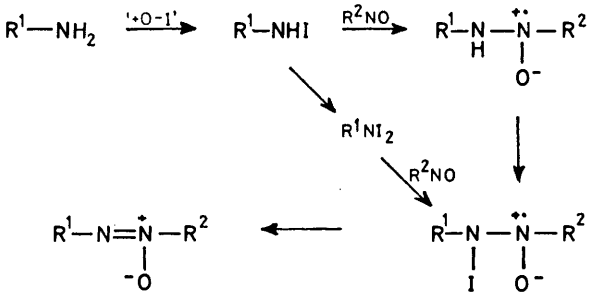
The realisation of such a process necessitated the development of a high-yielding unambiguous synthesis of unsymmetrical azoxyalkanes. From initial observations by Kovacic [6] it was apparent that condensation of dichloroamines and nitroso-compounds in the presence of potassium hydroxide led to low yields of azoxyalkanes. While the present work was in progress the same group



SCHEME 2

reported an improved condensation by the addition of transition-metal salts or other initiators.[7] We reasoned, however, that in the presence of t-butyl hypoiodite,[8] generated *in situ*, formation of the very weak iodoamine bond would be followed by homolysis and radical-trapping by the nitroso-compound to yield the azoxy-derivatives (Scheme 3).

Thus, reaction of cyclohexylamine with t-butyl hypochlorite in the presence of iodine and nitrosobenzene furnished the desired *N*-cyclohexyl-*N'*-phenyldiazene *N'*-oxide (1) smoothly and in virtually quantitative yield. Application of the same sequence to 3α- and 3β-amino-cholestanes yielded the stereoisomeric phenyl-azoxy derivatives (2a) and (2b) in 79 and 62% yield, respectively. The primary alkyl amine, n-butyl-amine, also afforded the desired derivative (3) in 78% yield.



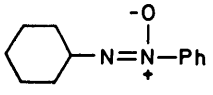
SCHEME 3

† Part 9, D. H. R. Barton, M. R. Britten-Kelly, and D. Ferreira, *J.C.S. Perkin I*, 1978, 1090.

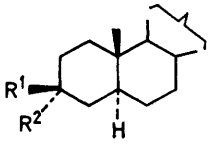
Having successfully modified and extended the scope of the condensation reaction, we then re-examined the reductive α-alkylation of azoxy-compounds by alkyl-lithium reagents. We were gratified to discover that reaction of (1) with an excess of butyl-lithium at −20 °C provided *N*-1-(1-n-butylcyclohexyl)-*N*′-phenyldiazene (4a) in 89% yield. Treatment with methyl-lithium led to the corresponding azo-derivative (4b) in 70% yield. Reduction of (4b) with zinc–acetic acid–ethanol gave 1-methylcyclohexylamine which was isolated in 69% yield and characterised as the benzoyl derivative.

Application of the same sequence to *N*-n-butyl-*N*′-phenyldiazene *N*′-oxide (3) resulted in the formation of 2-benzamidopentane in 41% overall yield. It is thus apparent that the reductive α-alkylation of azoxy-compounds is more useful than was originally realised.[5]

The stereochemistry of the alkylation was established by examining the reaction of the isomeric steroidal

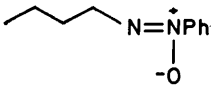


(1)

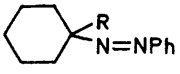


(2) a; R^1 = H, R^2 = N=$\overset{+}{N}$Ph, O^-

b; R^1 = N=$\overset{+}{N}$Ph, O^-, R^2 = H



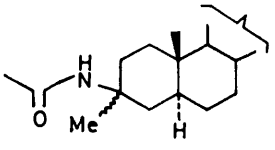
(3)



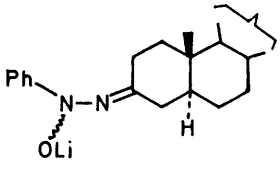
(4) a; R = Bu^n

b; R = Me

derivatives (2a and b). In each case, alkylation with methyl-lithium, followed by lithium–ethylamine reduction and subsequent acetylation of the crude product, led to the isolation of the same mixture of isomeric 3-methyl-3-acetamidocholestanes (5). Ritter reaction



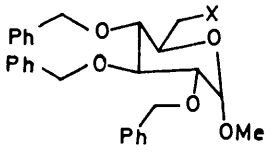
(5)



(6)

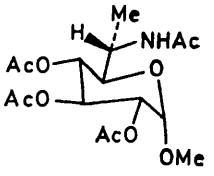
of 3-methylcholest-2-ene with acetonitrile confirmed the authenticity of the products and also formed a mixture of similar isomeric composition. These results are consistent with the intermediacy of an sp^2 hybridised α-carbon atom as in (6), which reacts with an alkyl-lithium with elimination of lithium oxide.

Application to the carbohydrate field was initiated by condensation of the aminoglycoside (7a) with nitroso-benzene to yield a crystalline azoxy-derivative (7b) in 65% yield. Reductive alkylation with methyl-lithium



(7) a; X = NH_2

b; X = N=$\overset{+}{N}$(O$^-$)–Ph



(8)

and sequential lithium–ethylamine reduction and acetylation of the crude product furnished, after careful column chromatography, a low yield of crystalline material. The analytical and spectroscopic data are clearly in accord with the α-methylated product (8). However, the 'unnatural' absolute stereochemistry depicted is suggested by the non-identity of the material with the degradation product of gentamicin B_1.[9]

EXPERIMENTAL

Melting points were determined with a Kofler hot stage apparatus. N.m.r. spectra were determined for solutions in [^{2}H]chloroform with tetramethylsilane as internal standard. Optical rotations were measured for solutions in chloroform. All solvents were purified and dried by standard techniques. Organic solvent extracts were dried over sodium sulphate.

N-*Cyclohexyl*-N′-*phenyldiazene* N′-*Oxide* (1).—t-Butyl hypochlorite (0.28 ml) was added rapidly to a stirred mixture of cyclohexylamine (100 mg), nitrosobenzene (120 mg), and iodine (600 mg) in benzene (10 ml), with cooling in an ice-bath. The mixture was allowed to warm to room temperature and stirring was continued for a further 16 h, after which time the reaction was quenched with aqueous sodium thiosulphate. The organic components were thoroughly extracted with ether, and the combined extract was washed with brine and dried. Evaporation of the solvent gave a crude product which was chromatographed on silica gel. Elution with benzene–light petroleum (1 : 2) gave the title compound (200 mg, 97%), whose spectral (i.r., n.m.r., and u.v.) and chromatographic properties (t.l.c.) were identical with those of an authentic sample.[7]

Repetition of this experiment with rigorous exclusion of light did not diminish the yield.

N-1-(*Methylcyclohexyl*)-N′-*phenyldiazene* (4b).—A solution of methyl-lithium in ether (5 ml; 1.7M) was added dropwise *via* syringe to a magnetically stirred solution of *N*-cyclohexyl-*N*′-phenyldiazene *N*′-oxide (100 mg) in ether (10 ml) under nitrogen with cooling in a bath of carbon tetrachloride–solid CO_2 (−20 °C). The reaction mixture was allowed to attain room temperature over 14 h and then quenched by the cautious addition of water. The aqueous phase was extracted with ether. The combined organic extracts were washed with water, brine, and dried. The solvent was evaporated off and the crude product was chromatographed on silica gel. Elution with benzene–

light petroleum (1 : 3) gave the desired compound (71 mg, 70%) which was characterised by reduction and benzoylation (see below).

N-*Benzoyl*-1-*methylcyclohexylamine*.—Zinc dust (8 g) was added portionwise over 1 h to a vigorously stirred solution of *N*-1-(1-methylcyclohexyl)-*N'*-phenyldiazene (500 mg) in acetic acid–ethanol (30 ml; 1 : 2). The reaction mixture was then heated under reflux for 3 h, cooled, and the excess of acid neutralised with sodium carbonate. The mixture was filtered and the residue washed with ether (2 × 10 ml). The solvent was carefully removed prior to treatment of a solution in pyridine of the crude amine thus obtained with benzoyl chloride (500 mg) at room temperature for 20 h. Dilution with ether, washing with dilute hydrochloric acid solution, 5% sodium hydrogen carbonate solution, water, and brine, and drying followed by removal of solvent gave a product which was chromatographed on neutral alumina. Elution with benzene–chloroform (3 : 1) yielded *N*-benzoyl-1-methylcyclohexylamine [10] (371 mg, 69%) whose spectroscopic (n.m.r., i.r.) and chromatographic properties (t.l.c.) were identical to those of an authentic sample. M.p. and mixed m.p. 101—102° (lit.,[10] 99—101°).

N-1-(1-*n-Butylcyclohexyl*)-N'-*phenyldiazene* (4a).—*N*-Butyl-lithium (1.8M in hexane; 5 ml) was added dropwise *via* syringe to a magnetically stirred solution of *N*-cyclohexyl-*N'*-phenyldiazene *N'*-oxide (100 mg) in ether (10 ml) under nitrogen at 0 °C. The mixture was allowed to warm to room temperature overnight and then quenched by the cautious dropwise addition of water. The aqueous phase was thoroughly extracted with ether, and the combined extracts were washed with water and brine, and dried. The solvent was evaporated off and the residue chromatographed on silica. Elution with benzene–light petroleum (1 : 1) gave the *title compound* (106 mg, 89%) as an oil, $\lambda_{max.}$ (cyclohexane) 268 nm (ε 10 120), *m/e* 244 (M^+) (Found: C, 78.5; H, 10.1; N, 11.5. $C_{16}H_{24}N_2$ requires C, 78.65; H, 9.9; N, 11.45%).

N-*n-Butyl*-N'-*phenyldiazene* N'-*Oxide* (3).—t-Butyl hypochlorite (0.28 ml) was added rapidly to a stirred mixture of n-butylamine (73.2 mg), nitrosobenzene (120 mg), and iodine (600 mg) in benzene (10 ml) with cooling in an ice-bath. The mixture was allowed to warm to room temperature over a 14 h period, after which time the reaction was quenched with aqueous sodium thiosulphate. The organic components were extracted with ether and the combined extracts washed with brine and dried. Removal of solvent and column chromatography gave the desired azoxy-compound (3) (142 mg, 80%), whose spectral (i.r., n.m.r., and u.v.) and chromatographic properties were identical with those of an authentic sample.[7]

2-*Benzamidopentane*.[11]—To a magnetically stirred solution of *N*-n-butyl-*N'*-phenyldiazene *N'*-oxide (100 mg) in ether (10 ml) under nitrogen was added *via* syringe methyllithium (5 ml; 1.7M in ether) with cooling in an ice-bath. The reaction mixture was allowed to warm to room temperature over 14 h. Work-up, zinc dust–acetic acid reduction, and benzoylation were carried out as previously described. The product was chromatographed on neutral alumina. Elution with benzene–chloroform (1 : 1) yielded the title benzamide (44 mg, 41%), identical with an authentic sample [m.p. and mixed m.p. 76—77° (lit.,[11] 76—78°)].

N-(5α-*Cholestan*-3β-*yl*)-N'-*phenyldiazene* N'-*Oxide* (2a).—t-Butyl hypochlorite (0.3 ml) was added rapidly to a stirred mixture of 3α-aminocholestane (380 mg), nitrosobenzene (170 mg), and iodine (600 mg) in benzene (10 ml) with cooling in an ice-bath. The reaction mixture was allowed to reach room temperature and stirring was continued for 21 h, before quenching with aqueous sodium thiosulphate. The aqueous phase was extracted with ether and the combined organic extracts were washed with water and brine and then dried. Removal of solvent *in vacuo* gave a solid (486 mg) which was purified by short-column chromatography on silica gel. Elution with benzene yielded the desired *azoxy-derivative* (2a) (381 mg, 79%). Recrystallisation from methanol–ether afforded white needles, m.p. 135.5—136°, $[\alpha]_D^{20}$ +20.8 (*c*, 1.45), $\nu_{max.}$ (CCl_4) 1 475 and 903 cm^{-1}, $\lambda_{max.}$ (cyclohexane) 246 nm (ε 10 020), τ 2.0 (2 H, m), 2.65 (3 H, m), and 5.62br (1 H, $W_{1/2}$ 7 Hz) (Found: C, 80.25; H, 10.7; N, 5.7. $C_{33}H_{52}N_2O$ requires C, 80.45; H, 10.65; N, 5.7%), *m/e* 492 (M^+).

N-(5α-*Cholestan*-3β-*yl*)-N'-*phenyldiazene* N'-*Oxide* (2b).—t-Butyl hypochlorite (0.3 ml) was added rapidly to a stirred mixture of 5α-cholestan-3β-ylamine (362 mg), nitrosobenzene (175 mg), and iodine (620 mg) in benzene (10 ml) with cooling in an ice-bath. The mixture was allowed to attain room temperature over 22 h and the reaction quenched with aqueous sodium thiosulphate. The aqueous phase was extracted with ether and the combined organic extracts were washed with water and brine, and dried. Removal of solvent *in vacuo* gave a solid (470 mg) which was purified by short-column chromatography on silica gel. Elution with benzene gave the *oxide* (2b) (287 mg, 62%), m.p. 145.5—146° (from ethanol–ether), $[\alpha]_D^{20}$ +11.1° (*c*, 1.43), $\nu_{max.}$ (CCl_4) 1 470 and 902 cm^{-1}, $\lambda_{max.}$ (cyclohexane) 247 nm (ε 10 800), τ 2.0 (2 H, m), 2.65 (3 H, m), 5.8br (1 H, $W_{1/2}$ 20 Hz) (Found: C, 80.15; H, 10.85; N, 5.6. $C_{33}H_{52}N_2O$ requires C, 80.45; H, 10.65; N, 5.7%), *m/e* 492 (M^+).

3-*Acetamido*-3-*methyl*-5α-*cholestane* (5).—Perchloric acid (60%; 0.3 ml) in acetonitrile (2 ml) and benzene (5 ml) was added dropwise to a magnetically stirred solution of 3-methyl-5α-cholest-2-ene (653 mg) in dichloromethane–acetonitrile (14 ml; 1 : 1) and stirring was continued for 20 h. The reaction was quenched with a saturated sodium carbonate solution and the aqueous phase was thoroughly extracted with chloroform. The combined organic extracts were washed with brine and dried and the solvent removed. Column chromatography on silica gel H-60 furnished a mixture of the desired isomeric acetamides (467 mg, 62%) as a viscous glass. By multiple running on analytical t.l.c. the presence of one major and one minor isomer was demonstrated, $[\alpha]_D^{19}$ +27° (*c*, 1.08), $\nu_{max.}$ (CCl_4) 3 450 and 1 675 cm^{-1}, τ 8.10 (3 H, s, Ac), 8.67br (3 H, s, Me) (Found: C, 81.5; H, 12.15; N, 3.15. Calc. for $C_{30}H_{53}NO$: C, 81.2; H, 12.05; N, 3.15%), *m/e* 433 (M^+).

3-*Acetamido*-3-*methyl*-5α-*cholestane* (5).—(a) *From* N-(5α-*cholestan*-3β-*yl*)-N'-*phenyldiazene* N'-*oxide*. Methyl-lithium in ether (1.7M; 2.5 ml) was added dropwise *via* syringe to a magnetically stirred solution of *N*-(5α-cholestan-3β-yl)-*N'*-phenyldiazene *N'*-oxide (117 mg) in ether (2.5 ml) under nitrogen with cooling in a bath of carbon tetrachloride–solid CO_2 (−20 °C). The mixture was allowed to warm to room temperature and stirring was continued for 16 h with protection from light. The reaction was quenched by the cautious addition of water, and the mixture was extracted thoroughly with ether, washed with brine, dried, and the solvent removed *in vacuo* to give a dark red oil (121 mg). This was reduced without purification.

Fresh finely-cut lithium shot (132 mg) was added in one portion to a stirred solution of the crude azo-compound

(121 mg) in freshly distilled ethylamine (5 ml). The mixture assumed and retained a dark blue colouration after 14 min. After a further 2 h, methanol was added to the cooled mixture and all solvents were then removed *in vacuo*. The crude product thus obtained was cooled in an ice-bath and treated with acetic anhydride (10 ml) and pyridine. After 16 h at room temperature the excess of reagents was removed *in vacuo* and the residue was taken up in diethyl ether–water. The aqueous phase was extracted with ether and the combined organic extracts were washed with dilute hydrochloric acid, aqueous sodium hydrogen carbonate, and brine, and dried. Removal of solvent gave a crude product (195 mg) which was purified by column chromatography on silica gel to give the stereoisomeric 3-acetamido-3-methylcholestanes (48 mg, 45%) as a viscous glass, $[\alpha]_D^{20}$ +29.1 (*c*, 0.2).

(b) *From* N-(5α-*cholestan*-3β-*yl*)-N′-*phenyldiazene* N′-*oxide*. Similar alkylation and reduction of *N*-(5α-cholestan-3β-yl)-*N*′-phenyldiazene *N*′-oxide afforded the stereoisomeric 3-acetamido-3-methylcholestanes (48%) as a viscous glass, $[\alpha]_D^{23}$ +29.5 (*c*, 0.3).

Careful spectroscopic (i.r., n.m.r., $[\alpha]_D^T$) and chromatographic (t.l.c.) comparison revealed that the same mixture of isomers was obtained from both the 3α- and 3β-phenylazoxy-steroids. The major stereoisomer produced in both these reactions is also the major isomer formed in the Ritter reaction.

N-6-(*Methyl* 2,3,4-*Tri*-O-*benzyl*-6-*deoxy*-α-D-*glucopyranosyl*)-N′-*phenyldiazene* N′-*Oxide* (7b).—t-Butyl hypochlorite (9.9 ml) was added rapidly to a stirred mixture of methyl 6-amino-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranoside (7a) [12] (3.94 g), nitrosobenzene (4.8 g), and iodine (19.8 g) in benzene (300 ml) with cooling in an ice-bath. After 17 h the reaction was quenched with saturated aqueous sodium thiosulphate. The organic components were extracted into benzene and the combined organic extracts washed with water and brine and dried. Evaporation yielded a product which was chromatographed on silica gel. Elution with hexene–chloroform gave the desired *azoxy-derivative* (7b) (3.02 g, 62.5%), m.p. 65—66° (from ether–light petroleum), $[\alpha]_D^{20}$ +58° (*c*, 1.0), $\nu_{max.}$ ($CHCl_3$) 1 470 and 1 290 cm^{-1}, $\lambda_{max.}$ 247 nm (ε 12 741), τ 2.7 (20 H, m), 6.61 (3 H, s,), and 4.9—6.4 (13 H, complex) (Found: (Found: C, 71.7; H, 6.6; N, 4.9; O, 16.6. $C_{34}H_{36}N_2O_6$ requires C, 71.8; H, 6.4; N, 4.95; O, 16.9%).

Methyl 6-*Acetamido*-2,3,4-*tri*-O-*acetyl*-6,7-*dideoxy*-β-L-glycero-D-gluco-*heptopyranoside* (8).—Methyl-lithium in ether (0.7M; 70 ml) was added dropwise with stirring to the azoxy-sugar (7b) (2.7 g) in ether (30 ml) under nitrogen with cooling in a bath of carbon tetrachloride–solid CO_2 (−20 °C). The mixture was allowed to warm to room temperature and stirring was continued for 16 h. The reaction was quenched by the cautious addition of water, extracted thoroughly with ether, washed with brine, and dried, and the solvent was removed *in vacuo* to give a dark red oil (2.3 g, 85.5%) which was reduced without purification.

Fresh, finely cut lithium shavings (1.2 g) were added rapidly to a stirred solution of the crude azo-compound (2.3 g) in freshly distilled anhydrous ethylamine (25 ml). After 45 min the reaction was quenched by the cautious addition of methanol, and all solvents were then removed *in vacuo*. The residue was treated with acetic anhydride (21 ml) and pyridine (7 ml) with cooling in an ice-bath and stirring was continued at room temperature for 16 h. Unchanged reagents were removed *in vacuo* and the residue was diluted with water and thoroughly extracted with chloroform. The organic extracts were washed with brine and dried, and the solvent was removed to give a dark oil which was purified by chromatography on silica gel. Elution with chloroform yielded a fraction (605 mg, 40%) whose n.m.r. spectrum suggested that it contained principally the desired alkylated derivative. A second chromatography on silica gel with sacrificial cuts yielded the pure *compound* (8) (100 mg, 6.5%), m.p. 193—194° (from diethyl ether), $[\alpha]_D^{20}$ +97° (*c*, 1.0), $\nu_{max.}$ ($CHCl_3$) 3 480, 1 750, and 1 670 cm^{-1}, τ 8.75 (3 H, d, *J* 5.8 Hz), 8.04 (6 H, s), 7.95 (3 H, s), 7.92 (3 H, s), 6.64 (3 H, s), 5.12 (1 H, s), and 4—6.6 (5 H, complex), *m/e* 375 (M^+) (Found: C, 51.3; H, 6.95; N, 3.6; O, 38.4. $C_{16}H_{25}NO_9$ requires C, 51.2; H, 6.7; N, 3.75; O, 38.35%).

Comparison of Synthetic and Authentic Samples.—Acetylation of an authentic sample of the triol derived by degradation of gentamicin B_1 gave a substance whose R_F value in several solvents corresponded with the synthetic material. Preparative t.l.c., however, yielded a solid, m.p. 118°, which was clearly different.

Conversely, hydrolysis of the synthetic triacetate (8) yielded an *N*-acetyl triol whose t.l.c. and spectral properties were different to those of the authentic specimen.

These conclusions were kindly confirmed by Dr. P. J. L. Daniels and Mr. Jay Weinstein of the Schering–Plough Corporation. We thank the Schering–Plough Corporation for their generous support of this work in its early stages. We also thank Dr. P. J. L. Daniels for authentic specimens.

[8/1412 *Received*, 31*st July*, 1978]

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