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Steroids. XIX. (1) 3-Phenyl- and 3-Ethyl-4-azacholestanes (2,3)

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The hydrogenation of 4-methyl-4-aza-5-cholesten-3-one (III) to yield the 5α- and 5β-isomers under acid conditions is described. The reaction of III and 4-methyl-4-aza-5α-cholestan-3-one with phenylmagnesium bromide, ethylmagnesium iodide, phenyllithium and ethyllithium to yield 3-substituted steroids is reported. Hydrogenation of these 3-substituted steroids yielded 4-methyl-3ξ-phenyl-4-aza-5α-cholestane and 3ξ-ethyl-4-methyl-4-aza-5α-cholestane (XIII). XIII inhibits the biosynthesis of cholesterol.

A sizeable proportion of the drugs in use today are weak electrolytes containing a basic nitrogen. The biological activity of these drugs is often dependent upon the presence of this nitrogen atom. The presence of a basic nitrogen in some steroids may result in useful and perhaps unusual biological activities.

Most of the reported azasteroids are lactams. A few of these lactams have been reduced to bases with sodium and amyl alcohol (6) or lithium aluminum hydride (7-12). This paper reports the synthesis of steroid bases by the reaction of N-alkyl lactams with Grignard type reagents. The reaction between Grignard reagents and N-methyl-2-pyrrolidone (13-15) and N-methyl-2-piperidone (16,17) yield products of this type, represented by I and II, respectively.

The reactions of phenylmagnesium bromide, ethylmagnesium iodide, phenyllithium, and ethyllithium with 4-methyl-4-aza-5-cholesten-3-one (III) and 4-methyl-4-aza-5α-cholestan-3-one (IV) were investigated in this laboratory. The yields with the Grignard reagents and lithium derivatives were 22-64% and 53-70%, respectively.

The hydrogenation of the 5,6-double bond in steroids has been shown to yield the 5α-isomer for steric reasons (18). Hydrogenation of III in ethanol or acetic acid, with platinum or Raney nickel as the catalyst, yielded IV as the only product. The reaction rate was greatest with platinum as the catalyst and acetic acid as the solvent. The structure of IV was confirmed by reduction with lithium aluminum hydride to 4-methyl-4-aza-5α-cholestane (V), a known compound (19).

Hydrogenation of III in glacial acetic acid in the presence of perchloric acid yielded an approximate 50:50 mixture of IV and 4-methyl-4-aza-5β-cholestan-3-one (VI). These products were separated as perchlorate salts. The structure of VI was confirmed by reduction with lithium aluminum hydride to 4-methyl-4-aza-5β-cholestane (VII), a known compound (19). This mixture of reduced lactams was anticipated since the addition of a strong acid to III might be expected to produce a salt (XIV) with a 4,5-double bond. The double bond in the enamine, 4-methyl-4-aza-5-cholestene, has been shown to shift to position 4 when an acid salt is prepared (7). Hydrogenation of the 4,5-double bond has been shown to lead to a mixture of 5α- and 5β-isomers (18).

The reaction of phenylmagnesium bromide with III and IV gave 4-methyl-3-phenyl-4-aza-2,5-cholestadiene (VIII) and 4-methyl-3-phenyl-4-aza-5α-cholest-2-ene (X) in 50 and 64% yields, respectively. No evidence of the presence of intermediate 3-hydroxy-3-phenyl- derivatives in the reaction products could be found with infrared spectra. VIII and X were isolated as light yellow crystalline solids which became a deep orange color, as a result of oxidation, upon exposure to air for a few hours. These compounds were stable when stored in a nitrogen atmosphere.

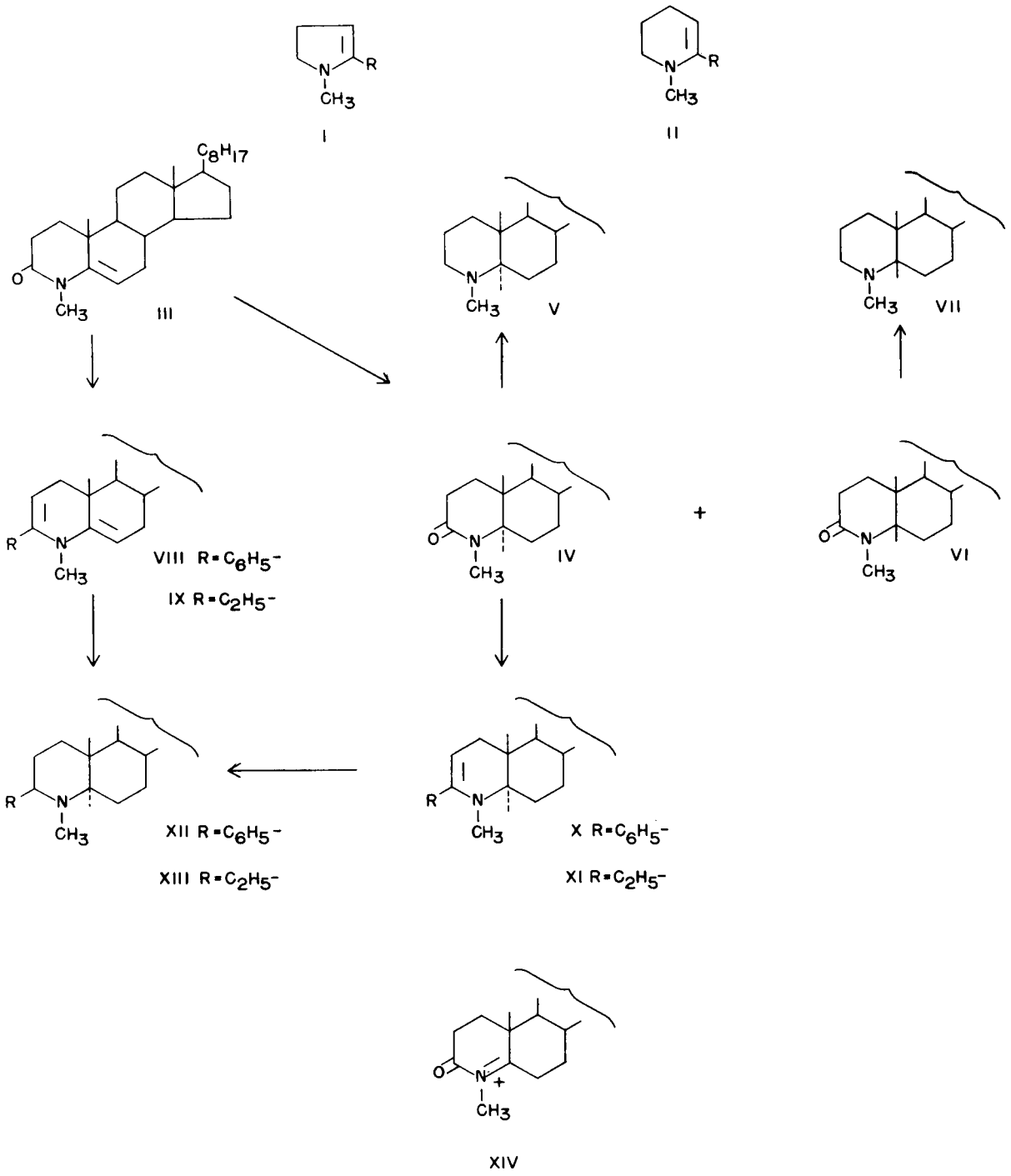
The reaction of ethylmagnesium iodide with III and IV gave 3-ethyl-4-methyl-4-aza-2,5-cholestadiene (IX) and 3-ethyl-4-methyl-4-aza-5α-cholest-2-ene (XI) in 22 and 38% yields respectively. Each of the reaction products, before purification, absorbed weakly at 2.72 μ suggesting the presence of the intermediate 3-hydroxy-3-ethyl- derivatives. Attempts to isolate these intermediates in pure form failed. IX and XI were stored in a nitrogen atmosphere since they were oxidized to orange and yellow products, respectively, upon exposure to air.

Previous investigators have not reported the reaction of organic lithium compounds with lactams. In this laboratory, phenyllithium and ethyllithium gave higher yields and less colored products than the corresponding Grignard reagents. The organic lithium compounds gave yields of 70% for VIII, 61% for X, 53% for IX and 65% for XI.

VIII and X yielded the same product, 4-methyl-3ξ-phenyl-4-aza-5α-cholestane (XII) upon hydrogenation in ethanol with either a platinum or Raney nickel catalyst. The yields were approximately 80%. Only one product was isolated from these reactions. The product appears to consist of a single isomer since no separation occurred when the product was chromatographed on alumina. We believe that it is the 3β-isomer but have not established this configuration.

The hydrogenation of IX and XI under similar conditions yielded 3ξ-ethyl-4-methyl-4-aza-5α-cholestane (XIII) in yields of 80-85%. XIII also appeared to be single isomer.

XIII lowered plasma levels of cholesterol by 30% when administered subcutaneously to male rats at 20 mg/Kg/day. Studies carried out with mice established that desmosterol accumulated in the liver indicating that the TPNH reduction of desmosterol was being inhibited (20).



EXPERIMENTAL (21)

4-Methyl-4-aza-5α-cholestan-3-one (IV).

Platinum oxide (0.5 g.) was added to a solution of 5.0 g. of 4-methyl-4-aza-5-cholesten-3-one (III) (7) in 200 ml. of acetic acid. The solution was treated with hydrogen at 60 lb. pressure and 70° for four hours. The catalyst was filtered and the solvent removed to yield 4.7 g. (94%) of IV as white needles, m.p. 112-114°. Two recrystallizations from petroleum ether (b.p. 30-60°) raised the melting point to 122-123°; $[\alpha]_D$ +23°; λ max 6.13 μ.

Anal. Calcd. for $C_{27}H_{47}NO$: C, 80.73; H, 11.80; N, 3.49. Found: C, 80.77; H, 11.82; N, 3.32.

4-Methyl-4-aza-5α-cholestane (V).

Three grams of IV were added to a slurry of 700 mg. of $LiAlH_4$ in 200 ml. of anhydrous ether and refluxed six hours. The mixture was hydrolyzed with water-saturated ether and water. The inorganic salts were filtered and washed with ether. The ether solutions were combined and dried over sodium sulfate. The solvent was removed and the residue was crystallized from methanol to yield 2.6 g. (89%) of V, m.p. 74-75° (reported 70-71°) (19); $[\alpha]_D$ +12° (reported +13°) (19); λ max no absorption 4.0-6.7 μ.

Anal. Calcd. for $C_{27}H_{49}N$: C, 83.64; H, 12.74; N 3.61. Found: C, 83.97; H, 12.80; N 3.22.

The *hydrochloride salt* of V was prepared, m.p. 293-294° (reported 268-274°) (19).

Anal. Calcd. for $C_{27}H_{50}NCl$: N, 3.33; Cl, 8.36. Found: N, 3.28; Cl, 8.35.

4-Methyl-4-aza-5α-cholestan-3-one (IV) and 4-Methyl-4-aza-5β-cholestan-3-one (VI).

4-Methyl-4-aza-5-cholesten-3-one (III) (7) (20.0 g.) and 20 ml. of 60% perchloric acid were dissolved in 200 ml. of acetic acid. Platinum oxide (0.5 g.) was added and the mixture was hydrogenated at 70° and 60 lb./sq. in. for 4 hours. The catalyst was filtered from the hot solution. After cooling the solution in an ice bath, a white crystalline solid separated which was filtered to obtain 13.5 g. (54%) of IV as the perchlorate salt, m.p. 240-242° dec. An analytical sample was obtained as white needles by crystallization from ethanol, m.p. 246-247° dec.

Anal. Calcd. for $C_{27}H_{48}O_5NCl$: N, 2.79; Cl, 7.06. Found: N, 3.09; Cl, 7.43.

The structure of this perchlorate salt was confirmed by converting a portion into the free lactam. One crystallization from petroleum ether yielded a crystalline solid, m.p. 122-123°, which was shown to be identical to IV by a mixed m.p. and comparison of infrared spectra.

The acetic acid filtrate from the hydrogenation reaction was evaporated to yield 11.5 g. (46%) of VI as the perchlorate salt, m.p. 170-172° dec. The salt was crystallized once from ethanol without a change in melting point.

Anal. Calcd. for $C_{27}H_{48}O_5NCl$: N, 2.79; Cl, 7.06. Found: N, 2.80; Cl, 6.76.

Five grams of this salt was treated with a mixture of aqueous sodium bicarbonate and ether. Evaporation of the ether gave a white solid, m.p. 100-107°. Crystallization from petroleum ether (b.p. 30-60°) yielded an analytical sample of 4-methyl-4-aza-5β-cholestan-3-one (VI); m.p. 107-109°; $[\alpha]_D$ +68°; λ max 6.12 μ.

Anal. Calcd. for $C_{27}H_{47}ON$: C, 80.73; H, 11.80; N, 3.49. Found: C, 80.98; H, 11.81; N, 3.24.

4-Methyl-4-aza-5β-cholestane (VII).

Two grams of VI were added to a slurry of 700 mg. of $LiAlH_4$ in 200 ml. of anhydrous ether and refluxed four hours. The mixture was hydrolyzed with water-saturated ether and water. The inorganic salts were filtered and washed with ether. The ether solutions were combined and dried over sodium sulfate. The solvent was evaporated to yield 1.8 g. (97%) of VII as an oil which resisted crystallization; $[\alpha]_D$ +30°; λ max no absorption 4.0-6.7 μ (reported as an oil; $[\alpha]_D$ +28°) (19).

The *hydrochloride salt* of VII was prepared and crystallized from acetone-ethanol to yield white platelets, m.p. 257-258° (reported 238-240°) (19).

Anal. Calcd. for $C_{27}H_{50}NCl$: N, 3.33; Cl, 8.36. Found: N, 3.33; Cl, 7.92.

4-Methyl-3-phenyl-4-aza-2,5-cholestadiene (VIII).

Method I.

To an ether solution of phenylmagnesium bromide, prepared from 0.73 g. of magnesium and 4.71 g. of bromobenzene, was added 8.00 g. of III. The mixture was refluxed 90 minutes and hydrolyzed with 100 ml. of 5% ammonium chloride solution while cooling in an ice bath. The ether layer was separated and dried over sodium sulfate. The residue obtained after removing the solvent was slurried with 30 ml. of acetone and filtered to yield 4.6 g. (50%) of VIII as light yellow crystals, m.p. 102-104°. An analytical sample was prepared by crystallizing from ethyl acetate; m.p. 104-105°; $[\alpha]_D$ -186°; λ max 244-252 mμ (log ε 4.06), 296 mμ (log ε 3.56), 6.07 μ and 6.23 μ.

Anal. Calcd. for $C_{33}H_{49}N$: C, 86.21; H, 10.74; N, 3.05. Found: C, 85.60; H, 10.83; N, 3.15.

Method II.

To an ether solution of phenyllithium, prepared from 0.21 g. of lithium and 2.35 g. of bromobenzene, was added 4.00 g. of III. The mixture was refluxed 90 minutes and worked up by the procedure described above to yield 3.2 g. (70%) of VIII as white crystals, m.p. 105-106°. The product was shown to be identical with that prepared by method I by a mixed melting point and a comparison of the infrared spectra.

3-Ethyl-4-methyl-4-aza-2,5-cholestadiene (IX).

Method I.

To an ether solution of ethylmagnesium iodide, prepared from 0.73 g. of magnesium and 4.7 g. of ethyl iodide, was added 8.00 g. of III. The mixture was refluxed 90 minutes and hydrolyzed by the addition of 100 ml. of 5% ammonium chloride solution while cooling in an ice bath. The ether layer was separated and dried over sodium sulfate. The residue obtained after removing the solvent was slurried with 30 ml. of acetone and filtered to yield 1.8 g. (22%) of IX as pale red crystals, m.p. 85-87°. An analytical sample was obtained as white needles by crystallization from ethyl acetate; m.p. 87-88°; $[\alpha]_D$ -112°; λ max 271 mμ (log ε 3.95), 5.87 μ and 6.02 μ.

Anal. Calcd. for $C_{29}H_{49}N$: C, 84.60; H, 12.00; N, 3.40. Found: C, 84.47; H, 12.15; N, 3.08.

Method II.

To an ether solution of ethyllithium, prepared from 0.42 g. of lithium and 3.27 g. of ethyl bromide, was added 4.0 g. of III. The mixture was refluxed 90 minutes and worked up by the procedure described for method I to yield 2.2 g. (53%) of IX as white crystals, m.p. 86-87°. This product was shown to be identical to the sample prepared by method I by a mixed melting point and a comparison of infrared spectra.

4-Methyl-3-phenyl-4-aza-5α-cholest-2-ene (X).

Method I.

To a solution of phenylmagnesium bromide, prepared from 0.36 g. of magnesium and 2.35 g. of bromobenzene, was added 4.00 g. of IV. The mixture was refluxed 90 minutes and worked up in the same manner as VIII to yield 3.0 g. (64%) of X, m.p. 106-108°. An analytical sample was obtained as white needles by crystallization from ethyl acetate; m.p. 110-111°; $[\alpha]_D$ -3°; λ max 216 mμ (log ε 4.05), 280 mμ (log ε 3.53), 6.08 μ and 6.23 μ.

Anal. Calcd. for $C_{33}H_{51}N$: C, 85.85; H, 11.13; N, 3.03. Found: C, 85.23; H, 11.07; N, 3.40.

Method II.

Two g. of IV was treated with phenyllithium in the same manner as in the preparation of VIII to yield 1.4 g. (61%) of X, m.p. 108-110°. The identity of the product was confirmed by a mixed melting point and comparison of infrared spectra with the product obtained by method I.

3-Ethyl-4-methyl-4-aza-5α-cholest-2-ene (XI).

Method I.

To an ether solution of 0.0075 mole of ethylmagnesium iodide was added 2.00 g. of IV. The mixture was refluxed 4 hours and worked up in the same manner as IX to yield 800 mg. (38%) of XI as an acetone insoluble solid, m.p. 60-64°, and 1.0 g. of the starting material IV, m.p. 114-117°. An analytical sample of the product was obtained as white needles by crystallization from ethyl acetate; m.p. 70-71°; $[\alpha]_D$ +3°; λ max 5.88 μ and 6.03 μ.

Anal. Calcd. for $C_{29}H_{51}N$: C, 84.18; H, 12.43; N, 3.39. Found: C, 83.91; H, 12.01; N, 3.68.

Method II.

To an ether solution of ethyllithium prepared from 0.21 g. of lithium and 1.63 g. of ethyl bromide, was added 2.0 g. of IV. The mixture was refluxed 90 minutes and worked up in the same manner as above to yield 1.35 g. (65%) of XI, m.p. 70-71°. The identity of this product was confirmed by a mixed melting point and comparison of infrared spectra with the product obtained by method I.

4-Methyl-3ξ-phenyl-4-aza-5α-cholestane (XII).

Method I.

A solution of 600 mg. of VIII in 250 ml. of ethanol was hydrogenated at room temperature and 45 lb./sq. in. in the presence of 100 mg. of platinum oxide. After 2 hours, the catalyst was filtered and the solvent evaporated to give a white solid, m.p. 151-153°. Crystallization from ethyl acetate yielded 480 mg. (79%) of XII as white needles; m.p. 156-157°; $[\alpha]_D$ +78°; λ max 6.23 μ.

Anal. Calcd. for $C_{33}H_{53}N$: C, 85.46; H, 11.52; N, 3.02. Found: C, 85.44; H, 11.52; N, 3.14.

Similar results were obtained when 100 mg. of Raney nickel was used as the catalyst.

Method II.

A solution of 600 mg. of X in 250 ml. of ethanol was hydrogenated at room temperature and 45 lb./sq. in. in the presence of 100 mg. of platinum oxide. After 4 hours, the catalyst was filtered and the solvent evaporated to give a white solid, m.p. 151-153°. Crystallization from ethyl acetate yielded 490 mg. (81%) of XII as white needles; m.p. 156-157°. This product was shown to be identical to XII prepared by the reduction of VIII by means of a mixed melting point and comparison of infrared spectra.

Similar results were obtained when 100 mg. of Raney nickel was used as the catalyst.

3ξ-Ethyl-4-methyl-4-aza-5α-cholestane (XIII).

Method I.

A solution of 600 mg. of IX in 250 ml. of ethanol was hydrogenated at 60° and 60 lb./sq. in. in the presence of 100 mg. of platinum oxide. After 2 hours, the catalyst was filtered and the solvent evaporated. The residue was crystallized from ethyl acetate to yield 490 mg. (81%) of XIII as white needles, m.p. 105-106°; $[\alpha]_D$ +46°; λ max no absorption 3.7-6.7 μ.

Anal. Calcd. for $C_{29}H_{53}N$: C, 83.78; H, 12.85; N, 3.37. Found: C, 83.76; H, 12.84; N, 3.54.

Similar results were obtained when 100 mg. of Raney nickel was used as the catalyst.

Method II.

A solution of 600 mg. of XI in 250 ml. of ethanol was hydrogenated at 60° and 60 lb. pressure in the presence of 100 mg. of platinum oxide. After 2 hours, the catalyst was filtered and the solvent evaporated. The residue was crystallized from ethyl acetate to yield 510 mg. (84%) of XIII, m.p. 105-106°. This product was shown to be identical to XIII prepared from IX by a mixed melting point and a comparison of infrared spectra.

Similar results were obtained when 100 mg. of Raney nickel was used as the catalyst.

REFERENCES

(1) For paper XVIII see N. J. Doorenbos and L. Milewich, *J. Org. Chem.*, in press.

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(6) C. C. Bolt, *Rec. trav. chim.*, 70, 940 (1951).

(7) N. J. Doorenbos and C. L. Huang, *J. Org. Chem.*, 26, 4106 (1961).

(8) S. Hara, *Pharm. Bull.* (Japan), 3, 209 (1955).

(9) B. M. Regan and F. N. Hayes, *J. Am. Chem. Soc.*, 78, 639 (1956).

(10) K. Tsuda and R. Hayatsu, *ibid.*, 78, 4107 (1956).

(11) S. Hara, *Yakugaku Zasshi*, 78, 1030 (1958).

(12) C. W. Shoppee and J. C. Sly, *J. Chem. Soc.*, 3458 (1958).

(13) J. C. Craig, *J. Am. Chem. Soc.*, 55, 295 (1933).

(14) J. C. Craig, *ibid.*, 55, 2543 (1933).

(15) R. Adams and J. E. Mahan, *ibid.*, 64, 2588 (1942).

(16) R. Lukes and O. Grossman, *Collection Czechoslov. Chem. Commun.*, 7, 533 (1936).

(17) J. Lee, A. Ziering, S. D. Heineman, and L. Berger, *J. Org. Chem.*, 12, 883 (1947).

(18) L. F. Fieser and M. Fieser, "Steroids", Reinhold Publishing Corp., New York, N. Y., 1959, pp. 271-274.

(19) J. McKenna and A. Tulley, *J. Chem. Soc.*, 945 (1960).

(20) Biological data furnished by Dr. J. Kerwin and Dr. Saunders of Smith Kline and French Laboratories.

(21) Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Specific rotations were determined on 1% solutions in $CHCl_3$ at 25°. U. V. spectra were obtained with a Spectracord on solutions in 95% ethanol. I.R. spectra were obtained with an Infracord using $CHCl_3$ solutions. Analyses were obtained from Drs. Weiler and Strauss, Oxford, England.

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