Acetylation of 17β-Acetoxy-5α-androstan-3-one[1]

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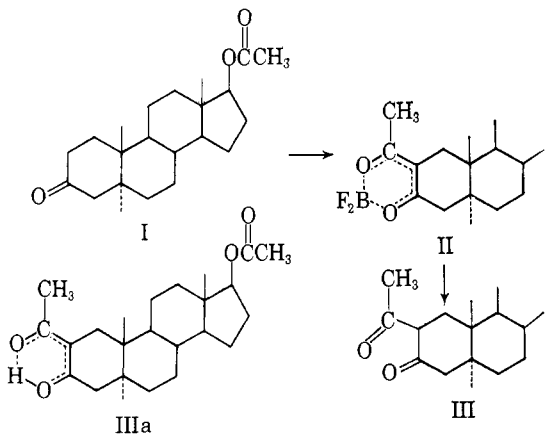
The familiar C-17 side chains of steroid hormones are acetyl or hydroxyacetyl functions. Marked changes in biological response usually accompany structural alteration in this side chain. We wished to investigate the effect of introducing the side chain usually associated with C-17 into position 2 of the steroid.

Syntheses of steroids substituted with the acetyl or hydroxyacetyl groups in positions other than C-17 have been reported, to our knowledge, in only a few instances. A dihydroxy acetone structure at and including C-3,[2a] 3-acetyl,[2b] and 16-acetyl and hydroxyacetyl[2c] steroids have been synthesized. To these, we wish to add the carbon acetylation of 17β-acetoxy-5α-androstan-3-one (I) by two methods, both on C-2.[2d]

Although C-acetylation of cyclohexanone and related ketones have been accomplished in low yields,[3] our many attempts to acetylate I by the alkaline condensation method were unsuccessful. It was by employing Hauser's inverse-addition method with acetic anhydride and boron trifluoride that we were able to obtain an acetylated product.[4] However, according to this method the boron fluoride complex is hydrolyzed with methanol and water, while the product we obtained following this treatment was the unhydrolyzed, acetylated steroid–boron fluoride complex (II). This was obtained in 73% yield and proved quite stable. The boron fluoride complex was characterized by strong



peaks in the infrared spectrum at 1733, 1592, and 1494 cm.$^{-1}$, and an ultraviolet absorption maximum at 307 mμ in methylene chloride. Even refluxing this complex in a biphasic system of ethylene chloride and aqueous acetate buffer yielded only starting material. It was by prolonged boiling of the complex in methanol that a 40% yield of 2-acetyl-17β-acetoxy-5α-androstan-3-one (III) was obtained. A superior method of decomposing the complex was to reflux a methanolic solution with sodium acetate and acetic acid for 2 hr. Upon cooling, the β-diketone crystallized and the yield was nearly quantitative.

From spectral data III appears to exist entirely in the cyclic, hydrogen-bonded, enolic form which is formulated as IIIa. It has an ultraviolet absorption peak at 290 mμ in 95% alcohol (ε 9600) and in the infrared a broad band at 1640–1570 cm.$^{-1}$, characteristic of enolic β-diketones, and a very weak peak at 2700 cm.$^{-1}$ (bonded hydroxyl). The ultraviolet peak of IIIa (290 mμ) occurs at a longer wave length than that reported for simple aliphatic β-diketones (*ca.* 270 mμ) or the closely related 2-formyl-3-keto steroids (*ca.* 282 mμ).[5] In basic solution both the 2-acetyl (III) and the 2-formyl derivatives exhibit maxima at 315 mμ. In a hydrochloric acid solution of III, there appeared a low intensity peak at 232 mμ in addition to the characteristic maximum at 290 mμ. As shown in Table I, the

TABLE I

MOLAR EXTINCTION OF 2-ACETYL-17β-ACETOXY-5α-ANDROSTAN-3-ONE

	0.01 *N* HCl	0.1 *N* HCl	1 *N* HCl
λ_{max} 232 mμ	2080	2190	2370
λ_{max} 290 mμ	9860	9770	8970

molar extinction of the former peak increased by about 12% in going from 0.01 *N* to 1 *N* acid while the 290-mμ peak decreased somewhat. The change in optical rotation (Δ[M]D) of +75° in going from the 3-keto steroid to the 2-acetyl-3-keto steroid is comparable with that observed in going from the same 3-keto steroid to the 2-formyl derivative (Δ[M]D +104°).[5]

An alternative synthesis of III was achieved through acylation of the enamine of I, with acetyl chloride. The pyrrolidine enamine (IV) of 17β-acetoxy-5α-androstan-3-one was conveniently prepared in 55% yield, according to the procedure of Heyl and Herr,[6] using benzene or toluene as solvent, a large excess of pyrrolidine, and without catalyst. Acylation of the enamine to 2-acetyl-17β-acetoxy-5α-androstan-3-one (III) was carried out with benzene or chloroform as solvent, the latter having the advantage of producing a homogeneous medium. Addition of triethylamine was found to increase the yield somewhat.

3-Keto 5α-steroids are known to enolize preferentially toward C-2.[7] That the acetylation took place on the 2-carbon rather than the 4-carbon in both syntheses was established by the following procedure: 2-acetyl-17β-acetoxy-5α-androstan-3-one (III) was treated with methyl iodide and potassium carbonate to yield 2-methyl-2-acetyl-17β-acetoxy-5α-androstan-3-one (V). The broad melting range, 128–177°, of this product

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(2) (a) H. B. Kagan, A. Marquet, and J. Jacques, *Bull. soc. chim. France*, 1079 (1960); (b) R. H. Baker and E. N. Squire, *J. Am. Chem. Soc.*, **70**, 1487 (1948); G. Nathansohn and O. Pirola, *Gazz. chim. ital.*, **90**, 407 (1960), and earlier papers; (c) D. Taub, R. D. Hoffsommer, H. L. Slates, and N. L. Wendler, *J. Org. Chem.*, **26**, 2852 (1961), and earlier papers; (d) A. J. Manson, F. W. Stonner, H. C. Neumann, R. G. Christiansen, R. L. Clarke, J. H. Ackerman, D. F. Page, J. W. Dean, D. K. Phillips, G. O. Potts, A. Arnold, A. L. Beyler, and R. O. Clinton [*J. Med. Chem.*, **6**, 1 (1963)] have also reported the synthesis of 2-acetyl-17β-acetoxy-5α-androstan-3-one.

(3) R. Levine, J. A. Conroy, J. T. Adams, and C. R. Hauser, *J. Am. Chem. Soc.*, **67**, 1510 (1945).

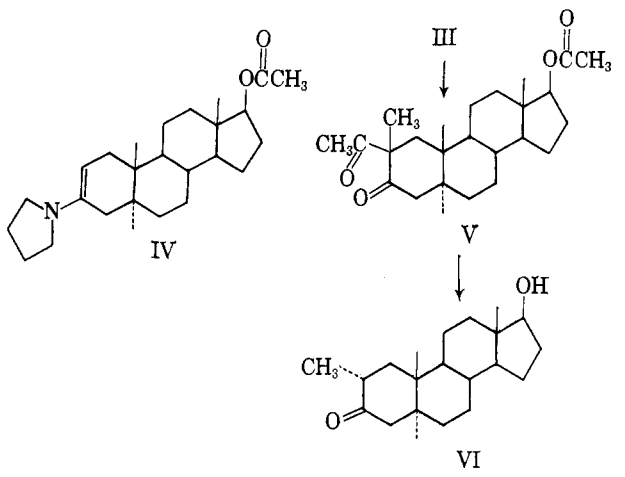
(4) C. R. Hauser, F. W. Swamer, and J. T. Adams, *Org. Reactions*, **8**, 129 (1954).

(5) R. O. Clinton, *et al.*, *J. Am. Chem. Soc.*, **83**, 1478 (1961).

(6) F. W. Heyl and M. E. Herr, *ibid.*, **75**, 1918 (1953).

(7) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 276.

indicated that it may be a mixture of the C-2 isomers. Cleavage of the acetyl group in base gave a single product which proved to be identical with an authentic sample of 2α-methyl-17β-hydroxy-5α-androstan-3-one (VI) from mixture melting point data and comparison of infrared spectra. Thus, the proton in C-2 rather than on C-4 appears to be removed, as is true in most instances with 3-keto 5α-steroids, although exceptions in special circumstances have been reported.[8]

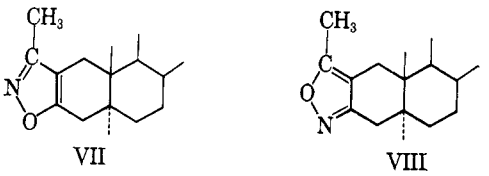


Acid hydrolysis of III at room temperature removed the 17-acetate. The resulting 2-acetyl-17β-hydroxy-5α-androstan-3-one, while having the characteristic broad β-diketone band at 1640–1540 cm.$^{-1}$, did not absorb in the "normal" carbonyl region from 1750 to 1700 cm.$^{-1}$. This apparent absence of the keto form, which was not evident before hydrolysis since the 17-acetate peak may have masked a ketone absorption peak in this region, was observed in chloroform and carbon tetrachloride. In contrast with this, two bands at about 1725 and 1700 cm.$^{-1}$ have been reported for a number of 1,3-diketones.[9] We have observed two such bands for 2-acetylcyclohexanone as well as some acyclic β-diketones. It would appear that the cyclic, hydrogen-bonded, enolic form is more stable in the 2-acetyl steroid than in 2-acetylcyclohexanone. This may be due to the greater conformational lability in the latter compound, resulting in greater tendency to open to the diketo form.

Bromine titration of III in methanol gave an average value of 87% enol. The higher proportion of ketone from titration results, as compared with spectral data, is consistent with the known shift of equilibrium toward the keto form with increasing solvent polarity.[10]

The copper chelate of III was prepared readily as a crystalline compound whose analysis revealed the expected metal–ligand ratio of 1:2. The infrared spectrum showed replacement of the "enol chelate" band with a strong but narrower band at 1578 cm.$^{-1}$ which has been characterized as due to a perturbed carbonyl.[9] A smaller band at 1530 cm.$^{-1}$ was evident, and such peaks have been assigned to perturbed carbon–carbon double bonds.

The isoxazole of III was prepared by a modification of the method of Clinton, *et al.*[11] The ultraviolet absorption maximum at 227 mμ (ϵ 6950) corresponds to the value obtained for 17β-hydroxy-5α-androstano-[2,3-*d*]isoxazole, 228 mμ (ϵ 4900), although the molar extinction coefficient of the latter is lower. The [2,3-*d*]isoxazole structure (VII) may appear to be favored over the [3,2-*c*]isoxazole (VIII); as yet, no clear structural assignment can be made on this or on molar rotation data.[12]



Experimental[13]

Borofluoride Complex of 2-Acetyl-17β-acetoxy-5α-androstan-3-one (II).—In a procedure patterned after Hauser's[4] a solution of 35 ml. of glacial acetic acid (0.611 mole) and 100 ml. of ethylene chloride in a 500-ml., three-necked flask equipped with a dropping funnel, gas-inlet tube, and stirrer was cooled in an ice bath. Boron trifluoride gas washed through sulfuric acid was passed into the stirred solution until saturation was reached. To the resulting white paste, kept under nitrogen, with continued cooling and stirring was added a solution of 21.8 g. (0.0755 mole) of 17β-acetoxy-5α-androstan-3-one and 28.7 ml. (0.306 mole) of acetic anhydride in 80 ml. of ethylene chloride. The addition required 15 min. during which time the mixture became homogeneous. After 0.5 hr. longer in the ice bath, the solution was allowed to stand at room temperature for 3 hr. under nitrogen. It was washed with water and saturated bicarbonate solution and dried, and the solvent was removed. Crystallization from acetone yielded 22.0 g. of white solid. With a second crop of 1.44 g. the combined yield was 73%. The melting range of a sample after recrystallization from acetone was 265–277°; $[\alpha]_D$ +38.7° (*c* 0.832); $\lambda_{max}^{CH_2Cl_2}$ 307 mμ (ϵ 12,200); infrared $\nu_{max}^{CHCl_3}$ 1733, 1592, 1494, 1156, 1052, 967, and 895 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{33}BF_2O_4$: C, 65.41; H, 7.88; F, 9.00. Found: C, 65.78; H, 8.14; F, 8.48.

2-Acetyl-17β-acetoxy-5α-androstan-3-one (III).—A mixture of 3.0 g. of the borofluoride complex II in 125 ml. of methanol containing 1.16 g. of sodium acetate and 0.8 ml. of glacial acetic acid was refluxed for 2.5 hr. Upon cooling and refrigerating, white crystals formed. These were filtered, washed successively with methanol and aqueous methanol, and finally dried to yield 2.52 g. (95%). Repeated crystallizations from methanol and/or acetone gave colorless needles with m.p. 180–181°; $[\alpha]^{28}D$ +43.3° (*c* 0.6116); λ_{max} 290 mμ (ϵ 9600); $\nu_{max}^{CCl_4}$ 2700 (very weak), 1748, 1640–1570 (broad), 1232, 1045, 1034, and 946 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 74.19; H, 9.34.

2-Acetyl-17β-acetoxy-5α-androstan-3-one (III) by the Enamine Method.—The pyrrolidine enamine (IV) was synthesized according to the procedure of Heyl and Herr[6] in 55% yield from 3.0 g. of I. Repeated crystallizations from ethyl acetate and methanol gave light yellow plates, the melting range of which remained broad (100–105°), $[\alpha]^{28}D$ +26.3° (*c* 0.96, chloroform) and +32.1° (*c* 1.06, methylene chloride). Neither solution

(8) Y. Mazur and F. Sondheimer, *J. Am. Chem. Soc.*, **80**, 6296 (1958).

(9) H. F. Holtzclaw, Jr., and J. P. Collman, *ibid.*, **79**, 3318 (1957).

(10) G. S. Hammond, W. G. Borduin, and G. A. Guter, *ibid.*, **81**, 4682 (1959).

(11) R. O. Clinton, A. J. Manson, F. W. Stonner, R. G. Christiansen, A. L. Beyler, G. O. Potts, and A. Arnold, *J. Org. Chem.*, **26**, 279 (1961).

(12) The structure proposed by Manson, *et al.*,[2d] corresponding to VII would not necessarily apply in this case, since they employed acidic conditions. The melting range which they report, 189–195°, indicates that their product is probably a mixture. Under our mildly alkaline conditions, we obtained predominantly one product. This pH effect appears to be the inverse of that found with isoxazole formation from 2-hydroxymethylene steroids in the above reference. However, it is more likely that only one isoxazole is formed in acid or base and the mixture obtained by Manson, *et al.*, is due to partial hydrolysis of the 17-acetate.

(13) The infrared data taken on a Perkin–Elmer Model 21 spectrophotometer are for major bands exclusive of the usual C–H stretching frequency (*ca.* 2900 cm.$^{-1}$) and the C–H deformation bands (1370–1470 cm.$^{-1}$). Ultraviolet spectra (in 95% ethanol unless otherwise indicated) were determined on a Cary Model 14 spectrophotometer.

showed significant change in rotation after 3 hr.; $\nu_{max}^{CH_2Cl_2}$ 1730, 1646, 1370, 1040, and 1032 cm.$^{-1}$.

Anal. Calcd. for $C_{25}H_{39}NO_2$: C, 77.87; H, 10.19; N, 3.63. Found: C, 77.85; H, 10.26; N, 3.68.

In a 100-ml., three-neck flask equipped with a condensor, dropping funnel, gas-inlet tube, and stirrer were placed 785 mg. (2.03 mmoles) of enamine IV, 235 mg. (2.32 mmoles) of triethylamine, and 10 ml. of chloroform, the latter two being freshly distilled and dried. To the stirred solution warmed to 40° and under nitrogen was added dropwise over a 20-min. period a solution of 167 mg. (2.13 mmoles) of acetyl chloride in 10 ml. of chloroform. The reaction temperature was raised to 55° for 4 hr., then lowered to 40° overnight.

The resulting orange-red solution was hydrolyzed with 30 ml. of 4 *N* hydrochloric acid and 20 ml. of chloroform by refluxing with vigorous stirring for 4 hr. The cooled mixture was extracted with methylene chloride; the organic layer was washed with water, dried, and evaporated to dryness, giving 620 mg. of orange-brown oil. A major portion of this, 580 mg., was chromatographed on 35 g. of silica gel (80–200 mesh). Following benzene, the eluate with 0.5% ethyl acetate–benzene yielded 152 mg. of III, identified after crystallization by melting point, mixture melting point, and infrared spectra. Further elution with 2% ethyl acetate–benzene gave 190 mg. of starting material (I).

The acylation of enamine IV in benzene followed a similar procedure with the difference that a white precipitate formed upon addition of acetyl chloride and persisted throughout the reaction. After hydrolysis with hydrochloric acid, 600 mg. of crude product was chromatographed on silica gel, yielding 135 mg. of III.

Methylation of 2-Acetyl-17β-acetoxy-5α-androstan-3-one (III).—To a solution of 2.0 g. of III in 75 ml. of acetone was added 4.0 g. of pulverized potassium carbonate and 7.0 ml. of freshly distilled methyl iodide. The mixture was refluxed with stirring for 20 hr., after which an additional 3 ml. of methyl iodide was added and the stirring with reflux was continued for 20 hr. more. After approximately half of the solvent was removed by distilling, the mixture was cooled and ether was added. The organic phase was washed with water, dried with magnesium sulfate, and evaporated to dryness. The 2.0-g. yield of crude product was chromatographed on 150 g. of silica gel (80–200 mesh). Elution with benzene was followed by several liters of 3% (v./v.) of ethyl acetate–benzene from which was obtained 1.64 g. of 2-methyl-2-acetyl-17β-acetoxy-5α-androstan-3-one (V). Homogeneity throughout the fraction was established by the identity of the melting points and spectra of samples from several aliquots. Repeated crystallizations from diisopropyl ether gave an analytical sample with m.p. 194–195°; $[\alpha]^{25}D$ +83.2° (*c* 1.01); $\nu_{max}^{CCl_4}$ 1738, 1710, 1235, 1124, and 1034 cm.$^{-1}$. There was no major absorption peak in the ultraviolet.

Anal. Calcd. for $C_{24}H_{36}O_4$: C, 74.20; H, 9.34. Found: C, 74.17; H, 9.16.

A solution of 383 mg. of V in 40 ml. of methanol containing 4.0 g. of potassium carbonate and 17 ml. of water was refluxed for 4 hr. under nitrogen. Most of the solvent was removed by evaporation under reduced pressure. To the remainder was added methylene chloride and the organic phase was extracted with aqueous potassium carbonate, washed with water, and dried with magnesium sulfate. Evaporation of the solvent gave 289 mg. (96%) of a white solid which, without purification, had melting point and spectral properties close to those of VI. Recrystallization from diisopropyl ether gave crystals with a melting point and mixture melting point (with an authentic sample) of 148–149°. Identity of infrared spectra was established in both chloroform and carbon disulfide.

Hydrolysis of 2-Acetyl-17β-acetoxy-5α-androstan-3-one (III).—A solution of 600 mg. of III in 50 ml. of 95% ethanol and 20 ml. of 6 *N* hydrochloric acid was refluxed 6.5 hr. Approximately half the ethanol was distilled and the remaining solution was cooled, treated with 70 ml. of water, and refrigerated overnight. The solid was filtered, washed well with water, and dried, yielding 504 mg. of a glassy solid. Crystallization from hexane gave an amorphous solid of broad melting range. Recrystallizations successively from aqueous methanol and ether–hexane gave well-formed platelets, m.p. 153–154°; $[\alpha]^{24}D$ +62.4°; $\nu_{max}^{CCl_4}$ 3620, 1640–1540 (broad), 1360, 1310, 1293, 1276, 1230, 1200, 1128, 1113, 1080, 1058, 1024, and 944 cm.$^{-1}$; ultraviolet absorption, λ_{max} 291 mμ (ε 8970).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.85; H, 9.51.

The same transformation was effected under mild conditions: a solution of 200 mg. of III in 30 ml. of methanol, 15 ml. of acetone, 5 ml. of water, and 2 ml. of concentrated hydrochloric acid was allowed to stand 64 hr. at room temperature. After addition of water, the mixture was extracted with methylene chloride and the latter phase was washed with dilute bicarbonate and water. Drying with magnesium sulfate followed by evaporation of solvent gave 177 mg. of crude material, which could be crystallized from petroleum ether to give semicrystalline material, m.p. 150–152°.

Bromine Titration of III.—The procedure followed was essentially that of Smith and Shriner.[14] To a solution of 0.463 mmole of III in 60 ml. of hot methanol was added an excess of bromine–methanol solution (*ca.* 0.1 *N*, freshly prepared) and followed immediately with 3 ml. of cyclohexene. An excess of potassium iodide solution was added; the mixture was warmed to 30–35°, and allowed to stand at room temprature for 10 min. After most of the iodine was titrated with 0.100 *N* sodium thiosulfate, there was added 2 ml. of acetic acid, 250 ml. of water, and 4 ml. of starch solution, and the titration was completed. A total of 8.15 ml. of thiosulfate solution was required, indicating an enol content of 88%. A repeat run gave a value of 86.6%.

Copper Chelate of III.—A solution of 500 mg. of III in 25 ml. of hot methanol was treated with cupric acetate solution [1 g. of $Cu(OAc)_2 \cdot H_2O$ in 10 ml. of hot water, filtered]. Dilution with water caused the precipitation of the green complex which was filtered and dried. Crystallization from acetone gave 427 mg. of green needles. From repeated crystallizations from acetone there was obtained an analytical sample, m.p. *ca.* 290° (began softening and darkening at *ca.* 270°); λ_{max} 243 mμ (ε 4100) and 311 (11,200); $\nu_{max}^{CHCl_3}$ 1738, 1578, 1530, 1467, 1378, 1290, 1023, and 948 cm.$^{-1}$.

Anal. Calcd. for $C_{46}H_{66}CuO_8$: C, 68.11; H, 8.21; Cu, 7.84. Found: C, 67.75; H, 8.18; Cu, 8.13.

Isoxazole Formation from III.—A solution of 900 mg. of III and 10 g. of hydroxylamine hydrochloride in 50 ml. of pyridine and 50 ml. of ethanol was refluxed for 2 hr. After cooling, dilution with water gave a precipitate which was filtered, washed, and dried. Crystallization from acetone gave 886 mg. of isoxazole, m.p. 201–203°. Recrystallization from acetone and hexane successively gave an analytical sample, m.p. 202–203°; $[\alpha]^{24}D$ +33.6° (*c* 0.708); λ_{max} 227 mμ (ε 6950); $\nu_{max}^{CCl_4}$ 1745, 1643, 1233, 1186, 1042, 1032, and 1021 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{33}NO_3$: C, 74.36; H, 8.95; N, 3.77. Found: C, 74.19; H, 8.97; N, 4.08.

(14) W. T. Smith and R. L. Shriner, "The Examination of New Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1956, p. 101.

The Reaction of Schiff Bases with Dicyandiamide. A New Synthesis of 4,6-Diamino-1,2-dihydro-*sym*-triazines

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4,6-Diamino-1-aryl-1,2-dihydro-*sym*-triazine hydrochlorides[2] (I, R = aryl) have previously been prepared by either the condensation of an arylamine hydrochloride, ketone or aldehyde and dicyandiamide, or by allowing the ketone or aldehyde to react with an arylbiguanide hydrochloride.[3] The latter method was ex-

(1) Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

(2) The tautomeric form indicated is done so arbitrarily. There is no evidence to date which favors this one over the other alternatives.

(3) (a) H. C. Carrington, A. F. Crowther, D. G. Davey, A. A. Levi, and F. L. Rose, *Nature*, **168**, 1080 (1951); (b) H. C. Carrington, A. F. Crowther, and G. J. Stacey, *J. Chem. Soc.*, 1017 (1954); (c) E. J. Modest, G. E. Foley, M. M. Pechet, and S. Farber, *J. Am. Chem. Soc.*, **74**, 855 (1952); (d) E. J. Modest, *J. Org. Chem.*, **21**, 1 (1956); (e) E. J. Modest and P. Levine, *ibid.*, **21**, 14 (1956).