The Adamantyl Group in Medicinal Agents. II. Anabolic Steroid 17β-Adamantoates

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The synthesis of several steroidal 17β-adamantoate esters is described. The adamantoic ester of 19-nortestosterone produces a profound and unique effect on anabolic potency as compared with other esters. The long duration of high myotropic activity with diminished androgenicity may be an indication that the ester is acting *in toto* as opposed to prior hydrolysis to the parent compound. The action of this anabolic ester over a period of several weeks after a single injection is confirmed by the radioactive aminoisobutyric acid (AIB) C^{14} uptake data.

There is a continuing search for compounds that will produce nitrogen retention. Interest stems from the challenge of separating the anabolic effect from other hormonal activities.

The nonandrogenic activities of testosterone and its derivatives in the stimulation of appetite, in producing weight gains, and imparting a sense of well being are well known. However, the androgenicity or ability to stimulate the sex accessory glands limits its practical use in many clinical cases. In recent years modifications of the androgenic steroids have produced potent steroids possessing primarily anabolic action with diminished androgenic activity. Esterification of the 17β-hydroxyl group in these steroids generally increases and prolongs the anabolic action.

In a thorough study of various esters of testosterone, Miescher, *et al.*,[1] found that while the lower acid esters, acetate, propionate, and formate, were of similar anabolic activity, the higher homologs, palmitic and stearic, were of long duration but of decreased intensity. The increase in duration of activity was not accounted for entirely by delayed absorption of the drug but also by protection from metabolism afforded by the long chain. Numerous esters of 19-nortestosterone, a potent anabolic agent, have been prepared and evaluated. These data are well documented in the excellent reviews by Junkman and Witzel[2] and also Camerino and Sala.[3] Newer ester derivatives include the *p*-hexoxyhydrocinnamate[4] and the *p*-chlorophenoxyacetate.[5] However, no complete dissociation of the androgenic effect has been observed to date.

The highly symmetrical cage-like adamantane molecule suggests lipophilic character, steric hindrance, and unique stability of its esters to chemical hydrolysis. Paper I of this series[6] reported the successful use of the adamantane moiety in medicinal agents as an N-alkyl substituent in the hypoglycemic N-arylsulfonyl-N′-alkylureas. The study of its effect in the ester portion of anabolic steroids is discussed in this report. The adamantane moiety in the form of adamantane-1-carboxylic acid (adamantoic acid) and its methyl and dimethyl derivatives were employed to produce esters of various C_{18} and C_{19} steroid 17β-hydroxy compounds.

Initially, the acids required for this study, adamantane-1-, 3-methyladamantane-1-, and 3,5-dimethyladamantane-1-carboxylic acids, were prepared from 1-bromoadamantane,[7] 1-bromo-3-methyladamantane, and 1-bromo-3,5-dimethyladamantane[6] by the established formic–sulfuric acids procedure of Stetter, *et al.*[8] Later, for larger amounts of adamantoic acid, the direct procedure of adamantane with *t*-butyl alcohol, sulfuric acid, and formic acid described by Koch and Haaf[9] was used. The corresponding acid chlorides were prepared by means of thionyl chloride[10] or PCl_5[8] and were employed *in situ* without extensive purification.

The esters were prepared by the action of the respective acid chloride with the steroidal alcohol in benzene solution in the presence of an equivalent amount of pyridine by the method described by Kuksis and Beveridge.[11] Other methods for esterification utilizing adamantoic anhydride[10] in pyridine or adamantoic acid-*p*-toluenesulfonic acid in benzene solution[12] have also been used. 4-Chloro-19-nortestosterone 17β-adamantoate (IX) was obtained from 19-nortestosterone adamantoate (VI) by epoxidation followed by hydrogen chloride treatment of the 4,5-oxide to yield the desired 4-chloro derivative.

The crystalline esters were isolated from the reaction mixture directly by recrystallization. In a few cases chromatography was employed to isolate the product. The steroids used included testosterone, dihydrotestosterone, and 19-nortestosterone derivatives. Esters of other steroid classes have also been prepared: estrogen 17β-adamantoates, adamantoates of 17α-hydroxyprogestins, and cortical 21-adamantoates; these will be reported subsequently.

The high degree of stability of these esters can be shown by the fact that hot 10% KOH–MeOH solution did not completely saponify the 19-nortestosterone adamantoate ester (VI) in 2 hr. while the cyclohexylcarboxylate ester was easily hydrolyzed in 1% solutions.

The assessment of potency and duration of effect were established by the myotropic–androgenic assay method of Hershberger, Shipley, and Meyer[13a] in im-

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(2) K. Junkman and H. Witzel, *Z. Vitamin-, Hormon-Fermentforsch.*, **9**, 222 (1957).

(3) B. Camerino and Sala, *Progr. Drug Res.*, **2**, 71 (1960).

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(11) A. Kuksis and J. M. R. Beveridge, *J. Org. Chem.*, **25**, 1219 (1960).

(12) E. Cataline, L. Worrell, S. Jeffries, and S. Aronson, *J. Am. Pharm. Assoc.*, **33**, 107 (1944).

(13) (a) L. Hershberger, E. Shipley, and R. Meyer, *Proc. Soc. Exptl. Biol. Med.*, **83**, 175 (1953). (b) The generic name approved for this compound is bidmantalate.

TABLE I

DURATION OF MYOTROPIC–ANDROGENIC RESPONSE TO ESTERS OF TESTOSTERONE (T) AND 2α-METHYLDIHYDROTESTOSTERONE (Me-DHT)

Compd.	Dose, mg.[a]	5 (7) days SV[b]	5 (7) days LA[b]	10 (14) days SV	10 (14) days LA	15 (18) days SV	15 (18) days LA	25 (28) days SV	25 (28) days LA
T adamantoate (I)	6.4	31.3	8.8	33.7	18.3	32.0	0.8	(29.0)	(−0.2)
4-Cl-T adamantoate (II)	10.0	1.7	6.7	1.4	9.3	(−0.6)	(−0.1)	−0.5	−11.1
T adamantoate-3-Me (III)	8.0	(12.8)	(1.6)	(38.4)	(−3.0)			(34.3)	(−0.8)
Me-DHT propionate	10.0	30.3	21.2	35.3	38.6	(22.8)	(21.1)	27.4	44.1
Me-DHT adamantoate (IV)	13.0	5.4	0.9	18.4	3.4	13.7	0.5	(3.5)	(7.2)
Me-DHT adamantoate-3-Me (V)	8.0	(2.8)	(5.4)	(0.7)	(14.4)			(−2.1)	(−13.0)

[a] Single subcutaneous injection in 0.2 ml. of either 0.5% carboxymethylcellulose or sesame oil. [b] Milligram increase of seminal vesicles and levator ani over control; average of 5 animals/group.

TABLE II

DURATION OF MYOTROPIC–ANDROGENIC RESPONSE TO ESTERS OF 19-NORTESTOSTERONE (NT)

Compd.	Dose, mg.[a]	5 (7) days SV[b]	5 (7) days LA[b]	11 (14) days SV	11 (14) days LA	18 (21) days SV	18 (21) days LA	25 (28) days SV	25 (28) days LA	48 days SV	48 days LA
NT adamantoate (VI)	8.1	12.0	18.1	9.3	23.9	5.5	30.4	9.4	73.8	9.6	73.1
NT adamantoate-3-Me (VII)	8.4	2.5	14.6	6.3	17.7	5.4	15.1	3.5	1.3	1.6	12.5
NT adamantoate-3,5-Me_2 (VIII)	8.7	3.8	7.3	4.9	3.4	1.5	6.1	1.3	−8.5	2.6	−1.4
4-Cl-NT adamantoate (IX)	8.0	(−0.8)	(−8.9)	(0.6)	(−1.6)			(4.6)	(−0.3)	−1.8	13.3

[a] Single subcutaneous injection in approximately 0.2 ml. of sesame oil. [b] Milligram increase of seminal vesicles and levator ani over control; average of 5 animals/group.

TABLE III

DURATION OF MYOTROPIC–ANDROGENIC RESPONSE TO LONG-ACTING ESTERS

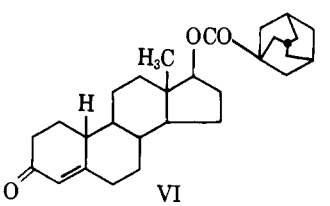
Compd.	Dose, mg.[a]	1 week SV[b]	1 week LA[b]	2 weeks SV	2 weeks LA	3 weeks SV	3 weeks LA	4 weeks SV	4 weeks LA	6 weeks SV	6 weeks LA	8 weeks SV	8 weeks LA
19-Nortestosterone adamantoate (VI)	8.0	5.5	26.4	6.8	36.9	11.2	58.1	18.8	75.4	8.8	103.6	9.4	97.3
19-Nortestosterone phenylpropionate (X)	7.5	100.8	39.9	137.9	65.1			82.6	69.8	3.6	3.7		
19-Nortestosterone *n*-decanoate (XI)	8.0	55.7	45.4	42.2	64.3	57.5	98.8	67.1	98.5	104.2	134.9	72.6	83.7
4-OH-19-nortestosterone cyclopentylpropionate (XII)	7.7	35.9	40.2	34.1	39.8	80.5	57.0	37.8	47.4				

[a] Single subcutaneous injection in approximately 0.2 ml. of sesame oil. [b] Milligram increase of seminal vesicles and levator ani over control; average of 5 animals/group.

mature castrate male rats. The data are reported in Tables I–III and Figure 1.

As seen in Table I, testosterone adamantoate (I) shows a good myotropic response as measured by levator ani muscle weight increases in 10–14 days while the 3-methyladamantoate ester (III) does not. Furthermore, the activity of I diminished rapidly by the 28th day. The anabolic activity of the 2α-methyldihydrotestosterone esters (IV and V), however, is greatly reduced. In the 19-nortestosterone series included in Table II, 19-nortestosterone adamantoate (VI) produced a highly significant levator ani response at all time periods studied, while at each time the seminal vesicle response was only minimal. The myotropic response persists beyond the 48-day test period. The 3-methyl- and 3,5-dimethyladamantoate esters (VII and VIII), however, are much lower in potency.

The effect of the unsubstituted adamantane esters is superior to that of the 3-mono or 3,5-dimethyl analogs both in regard to duration and intensity of effect. Similar results were evidenced in the N′-adamantyl-N-aryl-sulfonylureas[6] in which the unsubstituted adamantane moiety also produced the most active agent. From these data, it appears that the most appropriate steroid for esterification with adamantoic acid is 19-nortestosterone. In order to compare 19-nortestosterone 17β-adamantoate (VI)[13b] with



VI

several other long-acting esters, an anabolic study was followed for a period of 8 weeks after a single subcutaneous injection of the compounds described in Table III. For 19-nortestosterone adamantoate, the ratios of the levator ani to seminal vesicle responses at each time period were significantly higher than those of the other esters. The phenylpropionate ester (X) appears to peak in myotropic activity at 4 weeks, the decanoate ester (XI) in 6 weeks, and the cyclopentylpropionate (XII) in 3 weeks. Furthermore,

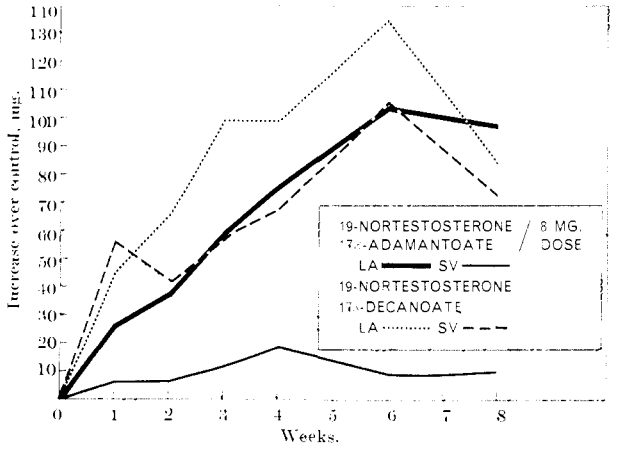


Figure 1.—Levator ani and seminal vesicle response to a single subcutaneous administration of steroid esters.

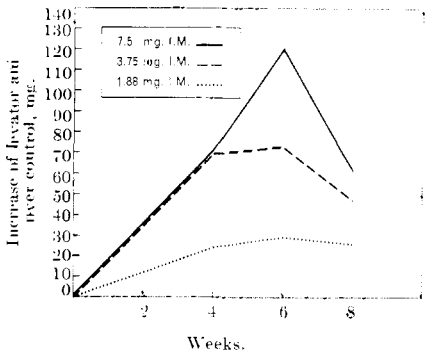


Figure 2.—Levator ani response to a single intramuscular administration of 19-nortestosterone 17β-adamantoate.

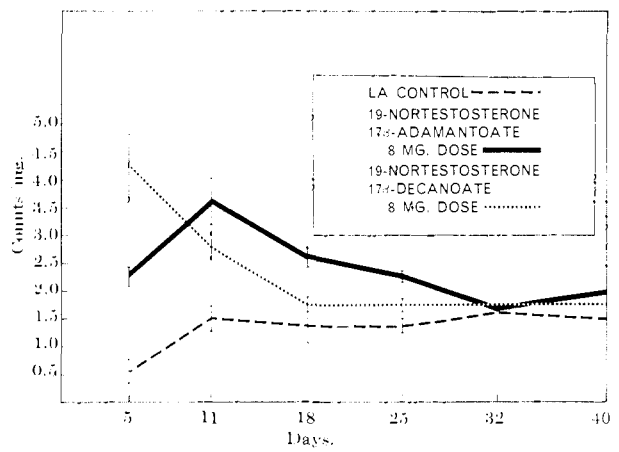


Figure 3.—Uptake of AIB-1-C^{14} into the levator ani at various times following a single subcutaneous injection of steroid esters.

the androgenicity, as measured by the seminal vesicle weight increases, is extremely low for VI as compared to the other esters, and the myotropic action of VI persists beyond 6 weeks. This sharp disassociation is illustrated in Figure 1. Since this degree of separation of myotropic and androgenic activities is not seen with 19-nortestosterone either by a single injection[14] or by repeated daily injections,[15] this suggests that this ester is not simply being hydrolyzed to the parent 19-nortestosterone.[15a]

A dose response following a single intramuscular administration is shown in Figure 2. With each of the three doses, the peak levator ani response is seen at 6 weeks, and significant elevation over controls persists at 8 weeks.

To determine the period of time that the adamantoate ester was actively influencing the target tissues, the uptake of radioactive aminoisobutyric acid-1-C^{14} (AIB) by the levator ani was evaluated by a modification (to be published) of the Metcalf and Broich method.[16] Illustrated in Figure 3 is the plot of the C^{14} counts incorporated per milligram of levator ani tissue of the treated and untreated groups *vs.* the period of days studied. The AIB C^{14} uptake thus substantiates the myotropic action of the adamantoate ester (VI) and furthermore indicates a long duration.

Experimental[17]

Adamantanecarboxylic Acids (Adamantoic Acids). Adamantoic acid and 3-methyl- and 3,5-dimethyladamantoic acid were prepared from the corresponding 1-bromoadamantane derivatives by the established formic-sulfuric acids procedure.[8] For larger quantities of adamantoic acid, the direct procedure of adamantane with *t*-butyl alcohol, H_2SO_4, and formic acid was used.[9]

Adamantoyl Chlorides.—The above carboxylic acids were converted to their respective acid chlorides by means of $SOCl_2$.[10] Adamantoyl chloride and its 3-methyl derivative have been reported previously.[8]

3,5-Dimethyladamantoyl chloride was similarly prepared using $SOCl_2$. Purification by distillation gave the acid chloride, b.p. 60–70° (7 mm.), infrared band at 5.55 μ, $n^{25}D$ 1.5017.

Anal. Calcd. for $C_{12}H_{19}ClO$: C, 68.86; H, 8.44. Found: C, 68.59; H, 8.44.

Steroid 17β-Adamantoates.—The preparation of the 17β-esters essentially followed a published procedure for steroid esters.[9] Physical constants for these compounds appear in Table IV. A typical esterification is described for 19-nortestosterone 17β-adamantoate (VI).

To a solution of 384 mg. (0.0014 mole) of 19-nortestosterone in 25 ml. of anhydrous benzene was added a solution of 332 mg. (0.0017 mole) of adamantoyl chloride in 10 ml. of anhydrous benzene followed by 0.2 ml. of dry pyridine. After refluxing for a period of 4 hr., the mixture was cooled and water and ether were added. The organic layer was washed with water and Na_2CO_3 solution and then dried (Na_2SO_4). Evaporation of the organic solvent yielded a crystalline residue (320 mg.), m.p. 200–205°. Recrystallization from ether provided the analytical sample, m.p. 203–206°, $[\alpha]^{25}D$ +53.36°.

The ester was also prepared utilizing adamantoic anhydride in pyridine solution,[10] as well as adamantoic acid *p*-toluenesulfonic acid in benzene solution,[12] but the yields were much lower.

4-Chloro-19-nortestosterone 17β-Adamantoate (IX).—To 2.61 g. (0.006 mole) of 19-nortestosterone 17β-adamantoate (VI) dissolved in 175 ml. of methanol while stirring at 0° were added dropwise and simultaneously solutions of 3.3 ml. of 4 *N* NaOH and 12.5 ml. of 30% H_2O_2 over a period of 8 min. The solution was stirred for an additional 55 min. at 0°. To this was added 0.85 ml. of glacial acetic acid, and the solution was poured into 700 ml. of salt solution. The mixture was extracted with three 100-ml. portions of ethyl acetate. The organic solution was washed with salt solution, dried (Na_2SO_4), and then evaporated to a white solid residue. Inspection of the ultraviolet spectrum indicated the absence of the conjugated ketonic system.

To the above epoxide dissolved in 50 ml. of chloroform was added 7.1 g. (0.06 mole) of pyridine hydrochloride, and the soln-

(14) G. Overbeek and J. deVisser, *Acta Endocrinol.*, **24**, 209 (1957).

(15) L. Barnes, R. Stafford, M. Guild, L. Thole, and K. Olson, *Endocrinology*, **55**, 77 (1954); R. Kraay, unpublished data.

(15a) NOTE ADDED IN PROOF.—Since this paper was submitted for publication, J. van der Vies [*Acta Endocrinol.*, **49**, 271 (1965)] has presented evidence that the phenylpropionate and decanoate esters of 19-nortestosterone are rapidly hydrolyzed *in vivo*. He concludes the ultimate substance affecting the levator ani muscle and seminal vesicles is 19-nortestosterone, and the difference in response to the esters is due to the rate at which the drug becomes available from the depot.

(16) W. Metcalf and J. Broich, *Proc. Soc. Exptl. Biol. Med.*, **107**, 744 (1961).

(17) All melting points are uncorrected. The microanalyses were performed by Messrs. W. L. Brown, G. M. Maciak, H. L. Hunter, and D. L. Cline.

TABLE IV

Compd.	Steroid	17β-Ester	M.p., °C.	Formula	% calcd. C	% calcd. H	% found C	% found H
I	Testosterone	17β-Adamantoate	217–220	$C_{30}H_{42}O_3$	79.95	9.39	79.73	9.44
II	4-Chlorotestosterone	17β-Adamantoate	304–306	$C_{30}H_{41}ClO_3$	74.27	8.51	74.31	8.59
III	Testosterone	3-Methyl-17β-adamantoate	168–170	$C_{31}H_{44}O_3$	80.13	9.55	79.55	9.96
	4-Bromotestosterone	17β-Adamantoate	222–224	$C_{30}H_{41}BrO_3$	68.04	7.80	68.85	8.04
	Δ^1-Androsten-(5α)-17β-ol-3-one	17β-Adamantoate	216–219	$C_{30}H_{42}O_3$	79.95	9.39	79.50	9.40
IV	2α-Methyl-5α-androstan-17β-ol-3-one	17β-Adamantoate	223–226	$C_{31}H_{46}O_3$	79.78	9.94	79.84	9.85
V	2α-Methyl-5α-androstan-17β-ol-3-one	3-Methyl-17β-adamantoate	209–211	$C_{32}H_{48}O_3$	79.95	10.07	80.13	10.35
VI	19-Nortestosterone	17β-Adamantoate	203–206	$C_{29}H_{40}O_3$	79.77	9.23	79.65	9.30
VII	19-Nortestosterone	3-Methyl-17β-adamantoate	159–161	$C_{30}H_{42}O_3$	79.95	9.39	79.83	9.40
VIII	19-Nortestosterone	3,5-Dimethyl-17β-adamantoate	132–134	$C_{31}H_{44}O_3$	80.12	9.54	80.35	9.65
IX	4-Chloro-19-nortestosterone	17β-Adamantoate	265–267	$C_{29}H_{39}ClO_3$	73.94	8.34	73.90	8.51

tion was allowed to reflux for 18 hr. After cooling, 100 ml. of chloroform and 50 ml. of water were added. The layers were separated, and the organic layer was extracted successively with water, 1% aqueous HCl, and water, and then dried (Na_2SO_4). Evaporation of the organic solvent yielded a residue which upon trituration with ether gave crystalline material (1.8 g.), m.p. 244–247°. Recrystallization from ether several times afforded the analytical sample, m.p. 265–267° dec., $[\alpha]^{25}D$ +66.55°.

Hydrolysis. A.—To 200 mg. of 19-nortestosterone 17β-cyclohexylcarboxylate dissolved in 20 ml. of methanol was added a solution of 0.2 g. of KOH in 0.5 ml. of water. After being allowed to reflux for 2 hr., the solution was cooled and then neutralized with glacial acetic acid. Water (20 ml.) was added, and the solution was extracted with three 100-ml. portions of ether. The combined ether solution was washed successively with dilute Na_2CO_3 solution and water, and was then dried (Na_2SO_4). Evaporation of the solvent afforded 162 mg. of a viscous residue which slowly crystallized. Thin layer chromatography (silica gel; chloroform–ether, 1:1) indicated the presence of 19-nortestosterone and the absence of 17β-ester.

B.—When 200 mg. of 19-nortestosterone 17β-adamantoate (VI) was treated in the identical manner as above, there was recovered, upon addition of the water, crystalline ester (190 mg.), m.p. 203–205°.

19-Nortestosterone 17β-adamantoate (200 mg.) in 20 ml. of methanol was treated with 2 g. of KOH in 1 ml. of water, and the solution was refluxed for 2 hr. Cooling, neutralization, and the addition of 20 ml. of water precipitated 105 mg. of ester, m.p. 193–197°. The filtrate was evaporated to a smaller volume to remove methanol, and the solution was then extracted with ether. The organic solution was washed with salt solution, dried (Na_2SO_4), and evaporated to a viscous residue (100 mg.). Thin layer chromatography showed that this residue consisted of 19-nortestosterone and some unhydrolyzed ester.

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Synthesis of α-(p-Aminophenyl)- and α-(p-Chlorophenyl)-β-arylpropionitriles by Catalytic Reduction of Stilbenenitriles

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Hydrogenation of α-(p-nitrophenyl)-β-arylacrylonitriles in the presence of palladium–charcoal in ethyl acetate solution gives α-(p-aminophenyl)-β-arylpropionitriles. The bearing of some of the results in this work upon earlier reductive cyclization of o-nitrophenylacetonitriles is discussed. Two compounds, α-(p-aminophenyl)-β-(3-pyridyl)propionitrile and the similarly prepared α-(p-chlorophenyl)-β-(3-pyridyl)propionitrile, affect adrenal cortical steroid secretion.

The 1,1-diaryl- and 1,2-diarylethylenes and -ethanes, notably stilbestrol and its relatives, have been of interest in endocrinology for some time.[1] Recently the field was given some new impetus by studies of adrenocortically active, similar basic ketones, the amphenones,[2–7] amphenone-related pyridyl ketones (*e.g.*, metyrapone and relatives),[8–10] and aminoindenes.[6] There also have been recent further studies of the well-known, weakly estrogenic stilbenenitriles[11] in the di-

(1) J. A. Hogg and J. Korman in "Medicinal Chemistry," Vol. 2, F. F. Blicke and C. M. Suter, Ed., John Wiley and Sons, Inc., New York, N. Y., 1956, Chapter 2.

(2) M. J. Allen and A. H. Corwin, *J. Am. Chem. Soc.*, **72**, 114, 117 (1950).

(3) W. L. Bencze and M. J. Allen, *J. Org. Chem.*, **22**, 352 (1957).

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(7) J. J. Chart and H. Sheppard, *ibid.*, **1**, 407 (1959).

(8) W. L. Bencze and M. J. Allen, *J. Am. Chem. Soc.*, **81**, 4015 (1959).

(9) W. L. Bencze, C. A. Burckhardt, and W. F. Yost, *J. Org. Chem.*, **27**, 2865 (1962).

(10) J. J. Chart, H. Sheppard, M. J. Allen, W. L. Bencze, and R. Gaunt, *Experientia*, **14**, 151 (1958).

(11) J. B. Niederl and A. Ziering, *J. Am. Chem. Soc.*, **64**, 885 (1942); see also S. Wawzonek, *ibid.*, **68**, 1157 (1946).