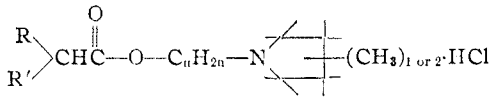
[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

Antispasmodics. V.[1] Mono- and Dimethylpyrrolidylalkyl Esters of Disubstituted Acetic Acids[2]

BY ROBERT BRUCE MOFFETT AND JAMES H. HUNTER

A series of thirty-seven new pyrrolidylalkyl esters is described in which one or two methyl groups are substituted in all possible positions of the pyrrolidine ring. Some of these exhibit very high antispasmodic activity.

TABLE I



R	R'	—C_nH_{2n}—N<(CH₃)₁ or ₂
C_6H_5	$CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—NCH(CH₃)$CH_2CH_2CH_2$
C_6H_5	$CH_2CH_2CH_2CH_2CH$—	—CH_2CH_2—NCH(CH₃)$CH_2CH_2CH_3$
C_6H_5	$CH_2CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—NCH(CH₃)$CH_2CH_2CH_2$
$CH_2CH_2CH_2CH_2CH$—	$CH_3CH_2CH_2$—	—CH_2CH_2—NCH(CH₃)$CH_2CH_2CH_2$
$CH_2CH_2CH{=}CHCH$—	$CH_3CH_2CH_2CH_2$—	—CH_2CH_2—NCH(CH₃)$CH_2CH_2CH_2$
$CH_2CH_2CH_2CH{=}CHCH$—	$CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—NCH(CH₃)$CH_2CH_2CH_2$
C_6H_5	$CH_2CH_2CH{=}CHCH$—	—$CH_2CH_2CH_2$—NCH(CH₃)$CH_2CH_2CH_2$
$CH_2CH_2CH_2CH_2CH$—	$CH_3CH_2CH_2$—	—$CH_2CH_2CH_2$—NCH(CH₃)$CH_2CH_2CH_2$
C_6H_5	$CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—$NCH_2CH(CH_3)CH_2CH_2$
C_6H_5	$CH_2CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—$NCH_2CH(CH_3)CH_2CH_2$
$CH_2CH_2CH_2CH_2CH$—	$CH_3CH_2CH_2$—	—CH_2CH_2—$NCH_2CH(CH_3)CH_2CH_2$
$CH_2CH_2CH_2CH{=}CHCH$—	$CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—$NCH_2CH(CH_3)CH_2CH_2$
C_6H_5	$CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—NCH(CH₃)₂$CH_2CH_2CH_2$
C_6H_5	$CH_2CH_2CH_2CH_2CH$—	—CH_2CH_2—NCH(CH₃)₂$CH_2CH_2CH_2$
C_6H_5	$CH_2CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—NCH(CH₃)₂$CH_2CH_2CH_2$
$CH_2CH_2CH_2CH_2CH$—	$CH_3CH_2CH_2$—	—CH_2CH_3—NCH(CH₃)₂$CH_2CH_2CH_2$
$CH_2CH_2CH_2CH{=}CHCH$—	$CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—NCH(CH₃)₂$CH_2CH_2CH_2$
C_6H_5	$CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—$NCH_2CH(CH_3)_2CH_2CH_2$
C_6H_5	$CH_2CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—$NCH_2CH(CH_3)_2CH_2CH_2$
$CH_2CH_2CH_2CH_2CH$—	$CH_3CH_2CH_2$—	—CH_2CH_2—$NCH_2CH(CH_3)_2CH_2CH_2$
C_6H_5	$CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—NCH(CH₃)$CH_2CH_2CHCH_3$
C_6H_5	$CH_2CH_2CH_2CH_2CH$	—CH_2CH_2—NCH(CH₃)$CH_2CH_2CHCH_3$
C_6H_5	$CH_2CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—NCH(CH₃)$CH_2CH_2CHCH_3$
$CH_2CH_2CH_2CH_2CH$—	$CH_3CH_2CH_2$—	—CH_2CH_2—NCH(CH₃)$CH_2CH_2CHCH_3$
C_6H_5	$CH_2CH_2CH{=}CHCH$—	—$CH(CH_3)CH_2$—NCH(CH₃)CH_2CH_2CHCH
C_6H_5	$CH_2CH_2CH_2CH{=}CHCH$—	—$CH(CH_3)CH_2$—NCH(CH₃)$CH_2CH_2CHCH_3$
$CH_2CH_2CH_2CH_2CH$—	$CH_3CH_2CH_2$—	—$CH(CH_3)CH_2$—NCH(CH₃)$CH_2CH_2CHCH_3$
C_6H_5	$CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—NCH(CH₃)$CH(CH_3)CH_2CH_2$
C_6H_5	$CH_2CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—NCH(CH₃)$CH(CH_3)CH_2CH_2$
$CH_3CH_2CH_2CH_2CH$—	$CH_3CH_2CH_2$--	—CH_2CH_2—NCH(CH₃)$CH(CH_3)CH_2CH_2$
C_6H_5	$CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—NCH(CH₃)$CH_2CH(CH_3)CH_2$
C_6H_5	$CH_2CH_2CH{=}CHCH$—	—$CH(CH_3)CH_2$—NCH(CH₃)$CH_2CH(CH_3)CH_2$
C_6H_5	$CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—$NCH_2CH(CH_3)CH(CH_3)CH_2$
C_6H_5	$CH_2CH_2CH_2CH_2CH$	—CH_2CH_2—$NCH_2CH(CH_3(CH(CH_3)CH_2$
C_6H_5	$CH_2CH_2CH_2CH{=}CHCH$—	—CH_2CH_2—$NCH_2CH(CH_3)CH(CH_3)CH_2$
$CH_3CH_2CH_2CH_2CH$—	$CH_3CH_2CH_2$—	—CH_2CH_2—$NCH_2CH(CH_3)CH(CH_3)CH_2$
$CH_2CH_2CH{=}CHCH$—	$CH_3CH_2CH_2CH_2$—	—CH_2CH_2—$NCH_2CH(CH_3)CH(CH_3)CH_2$

In continuation of the study of the relationship of structure to antispasmodic activity and in view of the high activity of some of the pyrrolidylalkyl esters previously reported,[3] it seemed desirable to prepare a number of such esters substituted on the

(1) The fourth paper in this series is: R. B. Moffett, J. H. Hunter and E. H. Woodruff, *J. Org. Chem.*, **15**, 1013 (1950).

(2) Reported before the XIIth International Congress of Pure and Applied Chemistry, New York, N. Y., September 10-13, 1951.

(3) H. G. Kolloff, J. H. Hunter, E. H. Woodruff and R. B. Moffett, THIS JOURNAL, **70**, 3862 (1948); *ibid.*, **71**, 3988 (1949); H. G. Kolloff, J. H. Hunter and R. B. Moffett, *ibid.*, **72**, 1650 (1950).

pyrrolidine ring. In this paper are reported esters in which one or two methyl groups are substituted in all the possible positions on the pyrrolidine ring disregarding stereoisomeric forms.

The esters were prepared by methods previously described[1,3] and are listed in Table I.

The requisite methyl-substituted pyrrolidyl alcohols were recently reported[4] except for 2-(2,2-dimethyl-1-pyrrolidyl)-ethanol, the preparation of which is to be included in a separate communication. The disubstituted acetic acids are for the

(4) R. B. Moffett, *J. Org. Chem.*, **14**, 862 (1949).

Free bases							Hydrochlorides						
Yield, %[a]	B.p. °C.	B.p. Mm.	$n^{25}D$	Empirical formula	Nitrogen, % Calcd.	Nitrogen, % Found[b]	Yield, %[c]	M.p., °C.[d]	Crystallizing solvent	Empirical formula	Chlorine, % Calcd.	Chlorine, % Found[b]	Antispasmodic activity[e]
88.2	150	0.2	1.5197	$C_{20}H_{27}NO_2$	4.47	4.67	82.8	126–128.5	EtOAc	$C_{20}H_{28}ClNO_2$	10.13	10.19	0.7
90.6	125	.02	1.5130	$C_{20}H_{29}NO_2$	4.44	4.39	80.4	133–136	MeEtCO + EtOAc	$C_{20}H_{30}ClNO_2$	10.08	10.06	.8
90.6	134	.03	1.5248	$C_{21}H_{29}NO_2$	4.28	4.20	76.1	146–148	MeEtCO	$C_{21}H_{30}ClNO_2$	9.74	9.54	.7
90.0	89	.02	1.4672	$C_{17}H_{31}NO_2$	4.98	4.77	93.2	119.5–120.5	EtOAc + Et_2O	$C_{17}H_{32}ClNO_2$	11.16	11.15	1.0
85.4[f]	103	.02	1.4735	$C_{18}H_{31}NO_2$	4.77	4.72	92.0	98.5–100	EtOAc + Et_2O	$C_{18}H_{32}ClNO_2$	10.75	10.65	0.3
81.3	130	.02	1.5022	$C_{20}H_{31}NO_2$	4.41	4.41	..	107–110	EtOAc	$C_{20}H_{32}ClNO_2$	10.02	10.06	.1
89.6	132	.01	1.5169	$C_{21}H_{29}NO_2$	4.28	4.47	83.3	166–167	EtOH + EtOAc	$C_{21}H_{30}ClNO_2$	9.74	9.68	.1
98.0	117	.03	1.4674	$C_{18}H_{33}NO_2$	4.74	4.83	82.0	167–169	EtOH + EtOAc	$C_{18}H_{34}ClNO_2$	10.68	10.65	.1
97.0	121	.01	1.5238	$C_{20}H_{27}NO_2$	4.47	4.54	60.8	102–106	EtOAc + Et_2O	$C_{20}H_{28}ClNO_2$	10.13	9.97	.1
93.5	133	.01	1.5238	$C_{21}H_{29}NO_2$	4.28	4.22	57.0	120–123	EtOAc + Et_2O	$C_{21}H_{30}ClNO_2$	9.74	9.60	.1
94.5	87	.01	1.4660	$C_{17}H_{31}NO_2$	4.98	4.99	83.0	96.5–98	EtOAc + Et_2O	$C_{17}H_{32}ClNO_2$	11.16	11.24	.1
83.4	126	.01	1.5012	$C_{20}H_{31}NO_2$	4.41	4.39	94.7	99–100	EtOAc + Et_2O	$C_{20}H_{32}ClNO_2$	10.02	9.86	.1
89.6	125	.015	1.5177	$C_{21}H_{29}NO_2$	4.28	4.29	83.4	132–142	EtOAc	$C_{21}H_{30}ClNO_2$	9.74	9.82	.7
74.0	140	.065	1.5109	$C_{21}H_{31}NO_2$	4.25	4.34	91.0	110–112	EtOAc	$C_{21}H_{32}ClNO_2$	9.69	9.63	.8
87.9	134	.014	1.5227	$C_{22}H_{31}NO_2$	4.10	4.12	67.5	157–158.5	MeEtCO	$C_{22}H_{32}ClNO_2$	9.38	9.45	.2
76.0	100	.03	1.4679	$C_{18}H_{33}NO_2$	4.74	4.72	88.9	119.5–121	EtOAc	$C_{18}H_{34}ClNO_2$	10.68	10.75	1.2
64.6	128	.013	1.5011	$C_{21}H_{33}NO_2$	4.23	4.32	73.0	130.5–132	EtOAc	$C_{21}H_{34}ClNO_2$	9.64	9.66	0.9
92.2	133	.04	1.5179	$C_{21}H_{29}NO_2$	4.28	4.27	89.5	116–118	EtOAc + Et_2O	$C_{21}H_{30}ClNO_2$	9.74	9.73	.02
92.2	134	.01	1.5180	$C_{22}H_{31}NO_2$	4.10	4.26	84.5	131.5–133.5	EtOAc	$C_{22}H_{32}ClNO_2$	9.38	9.41	.01
81.9	102	.04	1.4631	$C_{18}H_{33}NO_2$	4.74	4.80	72.2	107.5–108.5	EtOAc + Et_2O	$C_{18}H_{34}ClNO_2$	10.68	10.65	< .01
85.5	131	.02	1.5153	$C_{21}H_{29}NO_2$	4.28	4.41	67.5	117.5–122.5	EtOAc	$C_{21}H_{30}ClNO_2$	9.74	9.77	.6
92.3	138	.06	1.5089	$C_{21}H_{31}NO_2$	4.25	4.23	59.2	135.5–136.5	MeEtCO	$C_{21}H_{32}ClNO_2$	9.69	9.53	.3
90.2	145	.03	1.5202	$C_{22}H_{31}NO_2$	4.10	4.15	63.6	123–128	MeEtCO + EtOAc	$C_{22}H_{32}ClNO_2$	9.38	9.02	.6
81.2	113	.07	1.4657	$C_{18}H_{33}NO_2$	4.74	4.90	90.6	116–120	EtOAc	$C_{18}H_{34}ClNO_2$	10.68	10.86	.3
68.0	124	.015	1.5097	$C_{22}H_{31}NO_2$	4.10	4.11	44.0	143–146	EtOAc	$C_{22}H_{32}ClNO_2$	9.38	9.37	.01
77.1	154	.05	1.5147	$C_{23}H_{33}NO_2$	3.94	3.95	..	.....	.....	.....	...	...	< .01[g]
65.9	117	.03	1.4636	$C_{19}H_{36}NO_2$	4.53	4.61	..	.....	.....	.....	...	...	< .01[g]
80.5	138	.03	1.5170	$C_{21}H_{29}NO_2$	4.28	4.46	87.0	105–107.5	EtOAc + Et_2O	$C_{21}H_{30}ClNO_2$	9.74	9.78	.1
89.0	151	.04	1.5219	$C_{22}H_{31}NO_2$	4.10	4.19	58.6	145–156	MeEtCO	$C_{22}H_{32}ClNO_2$	9.38	9.53	.2
75.7	109	.03	1.4670	$C_{18}H_{33}NO_2$	4.74	4.72	88.3	101–104	EtOAc + Et_2O	$C_{18}H_{34}ClNO_2$	10.68	10.83	.05
..	132	.02	1.5147	$C_{21}H_{29}NO_2$	4.28	4.26	80.0	150–152	MeEtCO	$C_{21}H_{30}ClNO_2$	9.74	9.83	.1
..	121	.015	1.5078	$C_{22}H_{31}NO_2$	4.10	4.10	20.0	136–140	EtOAc + Et_2O	$C_{22}H_{32}ClNO_2$	9.38	9.29	< .01
93.5	126	.015	1.5133	$C_{21}H_{29}NO_2$	4.28	4.28	..	.....	.....	.....	...	...	.1[g]
91.6	125	.01	1.5065	$C_{21}H_{31}NO_2$	4.25	4.22	..	.....	.....	.....	...	...	< .01[g]
90.3	142	.02	1.5180	$C_{22}H_{31}NO_2$	4.10	4.08	72.0	122.5–124	EtOAc	$C_{22}H_{32}ClNO_2$	9.38	9.41	.01
86.5	95	.025	1.4634	$C_{18}H_{33}NO_2$	4.74	4.74	93.5	143–145	EtOAc	$C_{18}H_{34}ClNO_2$	10.68	10.50	.01
85.0[f]	124	.04	1.4696	$C_{19}H_{33}NO_2$	4.56	4.65	94.7	136–137.5	EtOAc	$C_{19}H_{34}ClNO_2$	10.31	10.41	< .01

[a] Unless otherwise noted the yield is based on the acid chloride. [b] Analyses by Mr. Harold Emerson and Staff of our Microanalytical Laboratory. [c] The yield is based on the distilled free base and would in most cases be essentially quantitative except that the filtrates from the crystallizations were not reworked. [d] Melting points are uncorrected. [e] Pharmacological assays were carried out by Dr. Milton J. VanderBrook and Mrs. E. K. Jordan in our Department of Pharmacology, on isolated rabbit intestine stimulated with acetylcholine chloride by the method of Magnus [*Arch. ges. Physiol.* (*Pflügers*), **102**, 123 (1904); *ibid.*, **103**, 515 (1904)]. The results are expressed as a fraction of the activity of atropine sulfate. [f] The yield is based on the acid used. The intermediate acid chloride was not isolated. [g] No crystalline salt was obtained. The free base was dissolved in dilute acid for the antispasmodic testing.

most part those found to give the most active antispasmodics.[1,3]

Preliminary pharmacological studies indicate that some of these compounds are among the most potent antispasmodics, so far reported, having an activity approximately equal to that of atropine sulfate. These results are listed in Table I.

KALAMAZOO, MICHIGAN RECEIVED OCTOBER 25, 1951

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S. A.]

Steroidal Sapogenins. XVI.[1a] Introduction of the 11-Keto and 11α-Hydroxy Groups into Ring C Unsubstituted Steroids (Part 3).[1b] 11-Oxygenated Sapogenins

BY CARL DJERASSI,[2] E. BATRES,[2] M. VELASCO AND G. ROSENKRANZ

The structure of 9α,11α-oxido-22-isoallospirostan-3β-ol-7-one acetate (IIa), the performic acid oxidation product of the corresponding $\Delta^{7,9(11)}$-diene I, was proved by converting it *via* the 7-cycloethylenemercaptol to the known 9α,11α-oxido-22-isoallospirostan-3β-ol acetate (IIc). Alkaline isomerization of the epoxyketone IIa led to $\Delta^{8(9)}$-22-isoallospirosten-3β,11α-diol-7-one (III), which after hydrogenation and Huang–Minlon reduction afforded 22-isoallospirostan-3β,11α-diol (Va). Oxidation to the corresponding 3,11-dione (Vc), followed by Raney nickel hydrogenation gave 22-isoallospirostan-3β-ol-11-one (Vd), while lithium aluminum hydride treatment of the dione yielded 22-isoallospirostan-3β,11β-diol (Vf). These experiments complete the partial synthesis of the three possible C-11 oxygenated 22-isoallospirostan-3β-ols from the abundant plant sapogenin, diosgenin. Lithium aluminum hydride reduction of 9α,11α-oxido-22-isoallospirostan-3β-ol-7-one acetate (IIa) affected only the 7-keto group and oxidation of the reduction product VIa followed by alkaline isomerization gave $\Delta^{8(9)}$-22-isoallospirosten-3,7-dione-11α-ol (VIIa), which was correlated with the original epxoyketone isomerization product IIIa by oxidation to $\Delta^{8(9)}$-22-isoallospirosten-3,7,11-trione (VIIc). Selective reduction of the 3-keto group of the latter produced $\Delta^{8(9)}$-22-isoallospirosten-3β-ol-7,11-dione acetate (VIII), which could also be obtained by the Fieser dichromate oxidation of $\Delta^{7,9(11)}$-22-isoallospirostadien-3β-ol acetate (I). Zinc dust reduction of the unsaturated diketone VIII yielded 22-isoallospirostan-3β-ol-7,11-dione acetate (IX).

C-11 oxygenated steroidal sapogenins represent almost ideal starting materials for the partial synthesis of cortisone and related adrenal steroids, but until now no such representative in the sapogenin series has been isolated from plant sources. The present paper is concerned with the partial synthesis of 11-keto (Vd), 11α-hydroxy (Va) and 11β-hydroxy (Vf) 22-isoallospirostan-3β-ols[3] from the abundant, ring C unsubstituted sapogenin Δ^5-22-isospirosten-3β-ol (diosgenin). The publication of the physical constants of the three 11-oxygenated 22-isoallospirostan-3β-ols (Va, d, f) should facilitate the identification of such sapogenins in the event that they should be encountered subsequently in plant sources. Furthermore, the presently described experiments represent an alternate route from diosgenin to cortisone, since 22-isoallospirostan-3β-ol-11-one (Vd), which has previously been synthesized from 22-isoallospirostan-3β-ol-12-one[1a] (hecogenin) as well as from diosgenin,[4] has already been transformed[4-7] into cortisone.

It has been reported[8] earlier from this Laboratory that performic acid oxidation of $\Delta^{7,9(11)}$-22-isoallospirostadien-3β-ol acetate (I)[9] affords 9α,11α-oxido-22-isoallospirostan-3β-ol-7-one acetate (IIa) and the structure of this key intermediate has now been proved through correlation with the known[10] 9α,11α-oxido-22-isoallospirostan-3β-ol acetate (IIc) by means of Raney nickel desulfurization of the 7-cycloethylenemercaptol IIb. The subsequent transformations of this epoxyketone IIa, which serve as additional structure proof, proceeded exactly as described[2,8] in the allopregnane series: alkaline isomerization led to $\Delta^{8(9)}$-22-isoallospirosten-3β,11α-diol-7-one (IIIa), which was hydrogenated[11] to the saturated 3β,11α-diol-7-one (IVa) and reduced by the Huang-Minlon[12] procedure to the desired 22-isoallospirostan-3β,11α-diol (Va). The spectral data of these intermediates, reported in the experimental section, fully support the structure assignments, while the α-configuration of the C-11 hydroxyl group (in IIIa, IVa and Va), and *ipso facto* of the 9,11-epoxide ring (in IIa, b, c), was demonstrated by the ease of acetylation (IIIb, IVb, Vb), characteristic[13] of the 11α- but not the 11β-hydroxy series.

The other two C-11 oxygenated 22-isoallospirostan-3β-ols (Vd, Vf) were prepared by unexceptional methods. The 3β,11α-diol Va was oxidized to 22-isoallospirostan-3,11-dione (Vc) and then hydrogenated with Raney nickel catalyst at room temperature, conditions which are not sufficient to reduce the C-11 keto group, to yield 22-isoallospirostan-3β-ol-11-one (Vd), identical with a specimen prepared[1a] from 22-isoallospirostan-3β-ol-12-one (hecogenin). An alternate synthesis of this 11-ketone Vd from diosgenin has already been re-

(1a) Paper XV, C. Djerassi, H. J. Ringold and G. Rosenkranz, THIS JOURNAL, **73**, 5513 (1951).

(1b) Part 2, C. Djerassi, O. Maucera, G. Stork and G. Rosenkranz, *ibid.*, **73**, 4496 (1951).

(2) Department of Chemistry, Wayne University, Detroit, Michigan.

(3) For nomenclature of steroidal sapogenins see G. Rosenkranz and C. Djerassi, *Nature*, **166**, 104 (1950). *Cf.* Report of Steroid Nomenclature Committee, *Helv. Chim. Acta*, **34**, 1680 (1951).

(4) E. M. Chamberlain, W. V. Ruyle, A. E. Erickson, J. M. Chemerda, L. M. Aliminosa, R. L. Erickson, G. E. Sita and M. Tishler, THIS JOURNAL, **73**, 2396 (1951).

(5) G. Rosenkranz, J. Pataki and C. Djerassi, *ibid.*, **73**, 4055 (1951).

(6) G. Rosenkranz, C. Djerassi, R. Yashin and J. Pataki, *Nature*, **168**, 28 (1951).

(7) J. M. Chemerda, E. M. Chamberlain, E. H. Wilson and M. Tishler, THIS JOURNAL, **73**, 4053 (1951).

(8) G. Stork, J. Romo, G. Rosenkranz and C. Djerassi, *ibid.*, **73**, 3546 (1951).

(9) The synthesis of this diene (I) from diosgenin has already been described [G. Rosenkranz, J. Romo, E. Batres and C. Djerassi, *J. Org. Chem.*, **16**, 298 (1951)].

(10) C. Djerassi, H. Martinez and G. Rosenkranz, *ibid.*, **16**, 1278 (1951).

(11) The smooth hydrogenation (palladized charcoal catalyst, room temperature, atmospheric pressure) is noteworthy if viewed in the light of the known resistance [*cf.* D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 214 (1949)] of $\Delta^{8(9)}$-stenols toward reduction, and indicates that it may possibly proceed through an enol form.

(12) Huang-Minlon, THIS JOURNAL, **71**, 3301 (1949).

(13) W. P. Long and T. F. Gallagher, *J. Biol. Chem.*, **162**, 511 (1946).