Structure–Activity Studies with the Selective Rat Toxicant Norbormide[1]

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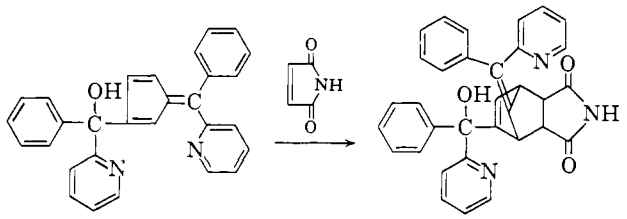
The preparation and lethality to rats of a series of norbormide analogs is given. The toxicity of norbormide stereoisomers is also presented. These stereoisomers were found to vary greatly in their potency. Substitution at any but the dicarboximide ring positions led to compounds less than one-twentieth as active as norbormide. Substitution on the imide nitrogen atom gave analogs varying in potency from less than one-twentieth norbormide to about the same potency as norbormide. None was more toxic to rats than norbormide. In general, rat toxicity was found to be highly dependent on structure.

The remarkably selective toxicity to genus *Rattus* of 5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (II, norbormide)[1a] has been reported.[2] In more than three dozen species of mammals (including other rodents), birds, and fish, norbormide was found to be nonlethal at doses 20–200 times the LD_{50} in rats.[2b]



I II, norbormide

Because of the utility of norbormide in the control of rats,[3] a study of the effects of varying structure on rat toxicity has been carried out in these laboratories.

Chemistry.—The synthesis of norbormide is described in our preliminary communication.[2a] Chemical investigation of norbormide and analogous compounds has proved to be relatively complex. In the synthesis of intermediate fulvenylmethanols (such as I) from diaryl ketones and cyclopentadiene, mixtures with 6,6-diarylfulvenes are often obtained.[4] In addition, the unsymmetrical fulvenylmethanols are obtained in two geometric forms, while reaction with maleimide introduces additional asymmetry. Thus, in the case of norbormide, eight racemic forms are possible.

In the synthesis of norbormide,[2a] five stereoisomers are formed in appreciable amounts. Other isomers are either formed in small amounts or can be produced by isomerization.[5] The stereoisomers of norbormide were isolated by laborious fractionation and chromatographic procedures.[5]

The substitution of various groups for the imide hydrogen atom of norbormide was accomplished either by treating fulvene I with an N-substituted maleimide when the latter was available (method A) or by the base-catalyzed N-alkylation of norbormide (method B). Representative procedures are given in the Experimental Section while results are summarized in Table I.

Other norbormide analogs (XXII–XXVIII) with changes of the aromatic groups and addition of a methyl group to the norbornene ring were obtained from the corresponding fulvenylmethanols[4] by reaction with maleimide as in the preparation of norbormide. The products are described in Table II. Compounds with a bromide atom or methyl group at the angular position were prepared by the reaction of I with the corresponding ring-substituted maleimide. As anticipated, these reactions proceeded with difficulty and the yields were low (Table II).

In some cases, crystalline products could not be obtained and therefore amorphous materials were characterized. Isolations were tedious due to the mixtures of stereoisomers. Although many of the preparations appeared to proceed cleanly, only modest yields of once-crystallized, wide-melting products were obtained in most cases. The sharper melting analytical samples were obtained in low yield. There was a tendency toward solvation in this series of compounds as well as difficulty in obtaining complete combustion during microanalysis. As a result, in the characterization considerable reliance was placed on thin layer chromatography (to show the absence of starting materials) and ultraviolet and infrared spectra. Proton resonance spectra were particularly useful in assigning general structures and in detecting the presence of solvents.

Structure–Activity Correlations.—Lethal doses of compounds were determined by oral administration of an aqueous suspension or dilute hydrochloric acid solution (where possible) to female white rats weighing 120–150 g. Initial surveys were made with three groups of three animals at 10-, 30-, and 100-mg/kg doses. For the more toxic analogs, a more exact LD_{50} was determined. Results are given in Tables I–III.

It can be seen that many analogs of norbormide were less than one-twentieth as potent as the parent compound. All changes of the aromatic rings, however slight, caused a marked decrease in activity. Even the substitution of a methyl group for the norbornene vinyl hydrogen atom (XXVIII) gave a product less than one-twentieth as toxic as norbormide. However, substitution of a methyl group at an angular position (XXX) only caused a partial loss of potency, while the

(1) (a) Norbormide is the American Standard Common Name for Shoxin®, the active ingredient of RATicate® rat killer. (b) Presented at the 149th National Meeting of the American Chemical Society, Detroit, Mich., April 1965.

(2) (a) A. P. Roszkowski, G. I. Poos, and R. J. Mohrbacher, *Science*, **144**, 412 (1964); (b) A. P. Roszkowski, *J. Pharmacol. Exptl. Therap.*, **149**, 288 (1965).

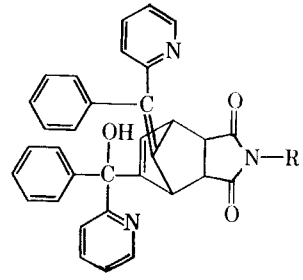
(3) D. G. Crabtree, W. H. Robison, and V. A. Perry, *Pest Control*, **32**, 36 (1964).

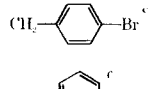
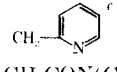
(4) R. J. Mohrbacher, V. Paragamian, E. L. Carson, B. M. Puma, C. R. Rasmussen, J. A. Meschino, and G. I. Poos, *J. Org. Chem.*, in press.

(5) R. J. Mohrbacher, H. R. Almond, Jr., E. L. Carson, J. D. Rosenau, and G. I. Poos, *ibid.*, in press.

TABLE I

NORBORMIDE AND N-SUBSTITUED ANALOGS

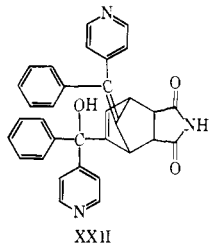


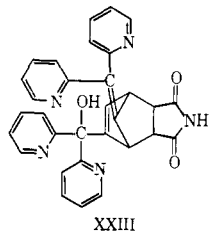
No.	R	Method of prepn	Mp, °C[a]	Solvent of crystn	Yield,[b] %	$\lambda_{max}^{CH_3OH}$, mμ (ε)[c]	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	LD_{50} (rats), mg/kg *po*[d]
II	H (norbormide mixed isomers)	...	190–198	Methylene chloride–ether	90	248 (16,500)	$C_{33}H_{25}N_3O_3$	77.48	4.93	8.22	77.61	5.05	7.92	5.3
III	CH_3[e]	A, B	209–211	Ethyl acetate	A-51 B-29	248 (17,100)	$C_{34}H_{27}N_3O_3$	77.69	5.18	8.00	77.80	5.31	8.05	12.0
IV	C_2H_5[e]	A	168–180	Ethyl acetate	40	250 (16,200)	$(C_{35}H_{29}N_3O_3)_2 \cdot C_3H_8O_2$[f]	76.14	5.70	7.20	76.28	5.76	7.39	7.5
V	n-C_3H_7[e]	A	198–200	Ethyl acetate	57	250 (18,000)	$C_{36}H_{31}N_3O_3$	78.10	5.64	7.59	78.30	5.24	7.87	34.5
VI	i-C_3H_7[e]	A	210–211	Ethyl acetate	44	248 (17,900)	$C_{36}H_{31}N_3O_3$	78.10	5.64	7.59	77.87	5.40	7.55	10–30
VII	n-C_4H_9[e]	A	171–172	Ethyl acetate–n-hexane	56	248 (18,200)	$C_{37}H_{33}N_3O_3$	78.28	5.86	7.40	78.12	5.56	7.24	9.0
VIII	sec-C_4H_9[e]	A	195–196.5	Ethyl acetate	28	248 (17,600)	$C_{37}H_{33}N_3O_3$	78.28	5.86	7.40	78.00	5.89	7.12	30–100
IX	i-C_4H_9[e]	A	196–198	Ethyl acetate	23	248 (17,600)	$C_{37}H_{33}N_3O_3$	78.28	5.86	7.40	78.36	6.00	7.51	30–100
X	t-C_4H_9[e]	A	192–193.5	Ethyl acetate	27	248 (17,600)	$C_{37}H_{33}N_3O_3$	78.28	5.86	7.40	78.34	6.12	7.40	>100
XI	$(CH_2)_7CH_3$[e]	A	159–160.5	Ethyl acetate	57	250 (18,000)	$C_{41}H_{41}N_3O_3$	78.94	6.63	6.74	79.07	6.75	6.78	30–100
XII	$CH_2CH{=}CH_2$[e]	B	157–188	Acetone	29	251 (18,500)	$(C_{36}H_{29}N_3O_3)_2 \cdot C_3H_6O$[g]	77.56	5.56	7.24	77.74	5.71	7.20	10–30
XIII	$CH_2CH_2OCH_3$[e]	A	172–173	CCl_4–petr ether	24	250 (17,900)	$C_{36}H_{31}N_3O_4$	75.90	5.49	7.38	76.10	5.50	7.23	9.0
XIV	$(CH_2)_3N(CH_3)_2$[e]	B	79–120[h]	Ether–petr ether	44	249 (17,500)	$C_{38}H_{36}N_4O_3$	76.48	6.08	9.39	76.14	6.33	9.21	30–100
XV	$(CH_2)_2N(CH_3)_2$[e]	B	131–148	Acetone–ether	24	249 (18,200)	$C_{37}H_{34}N_4O_3$	76.26	5.88	9.62	76.23	6.05	9.74	10–30
XVI	C_6H_5	A	225–227	Tetrahydrofuran–ether	30	251 (20,200)	$C_{39}H_{29}N_3O_3$	79.71	4.97	7.15	79.50	5.33	7.28	>100
XVII	C_6H_{11}[e]	A	198.5–200	$CHCl_3$–ether	78	248 (19,000)	$C_{39}H_{35}N_3O_3$	78.89	5.94	7.08	75.69[i] 77.92	5.84 5.95	6.54 6.90	>100
XVIII	$CH_2C_6H_5$[e]	A	200–201.5	$CHCl_3$–ether	40	250 (17,400)	$C_{40}H_{33}N_3O_3$	79.84	5.19	6.98	78.05[i] 79.15	5.21 5.29	6.60 7.06	>100
XIX		B	226–230	Dichloromethane–ethyl acetate	75	247 (18,400)	$C_{46}H_{30}BrN_3O_3$	70.51	4.44	6.17	70.16	4.44	6.04	>100
XX		B	110–172[h]	Ether–petr ether	36	251 (19,900)	$C_{39}H_{30}N_4O_3$	77.72	5.02	9.30	77.40	5.16	9.55	5.2
XXI	$CH_2CON(CH_3)_2$	B	128–140[h]	Ether–petr ether	65	248 (16,600)	$C_{37}H_{32}N_4O_4 \cdot H_2O$	72.29	5.58	9.12	72.13	5.57	9.02	5.9

[a] Melting points are of analytical samples. [b] Yields are of once crystallized or precipitated products. Because of the many isomers, isolations were tedious so that actual yields are probably higher than those shown. [c] Broad maxima which give multiple peaks at high concentration. [d] Milligram values for the LD_{50} were calculated by the method of J. T. Litchfield, Jr., and F. Wilcoxon, *J. Pharmacol. Exptl. Therap.*, **96**, 99 (1949). Other values are based on 2/3 or 3/3 animals at dose levels of 10, 30, and 100 mg/kg. [e] Only *endo* isomers by nmr spectrum.[5] [f] Ethyl acetate solvate by nmr. [g] Acetone solvate by nmr. [h] Amorphous powder. [i] Representative analyses from two independent laboratories on the same sample.

TABLE II

RING ANALOGS OF NORBORMIDE


XXII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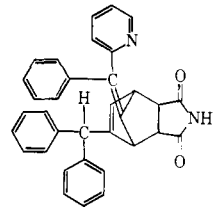
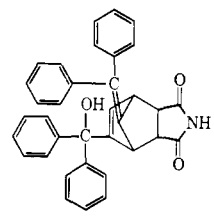
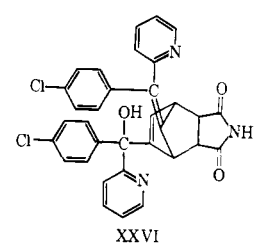

XXIV


XXV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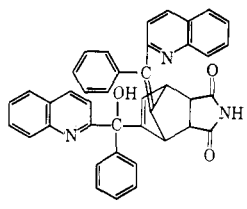
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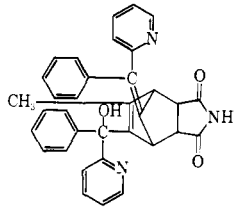
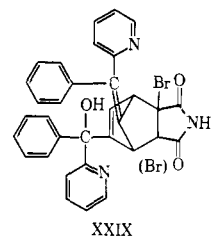
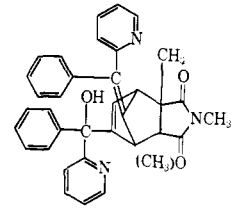

XXVIII


XXIX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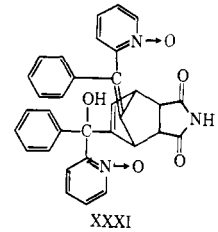
XXXI

No.	Mp, °C	Solvent of recrystn	Yield,[a] %	$\lambda_{max}^{CH_3OH}$, mμ (ε)[b]	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	Toxicity (rat), mg/kg po[c]
XXII[d]	270 dec	Ethanol	33	253 (13,500)	$C_{33}H_{25}N_3O_3 \cdot C_2H_5OH$[e]	75.38	5.60	7.54	75.24	6.03	7.53	>100
XXIII[d,f]	208-210	Xylene	53	249, 254, 260, 267 (18,500, 18,800, 18,800 17,500)	$C_{31}H_{23}N_5O_3$	72.50	4.51	13.64	72.60	4.74	13.51	>100
XXIV[d]	205-207	Ether–petr ether	27	245 (15,900)	$C_{34}H_{26}N_2O_2$	82.57	5.30	5.66	82.64	5.31	5.65	>100
XXV[d]	210-211	Ethyl acetate–cyclohexane	23	249 (16,800)	$C_{36}H_{27}NO_3$			2.75			2.78	>100
XXVI[g,h]	139–190	Methanol–water	13	224, 253 (25,600, 20,600)	$C_{33}H_{23}Cl_2N_3O_3$	68.28	3.99	7.24	68.25	3.68	7.28	>100
XXVII[g,h]	146-191	Benzene-*n*-hexane	57	233, 254 (78,500, 24,400). Fine structure: 289, 296, 302, 308, 316 (8000–11,400)	$C_{41}H_{29}N_3O_3$	80.50	4.78	6.87	80.61	5.45	7.05	>100
XXVIII	Isomer A[d,i] 226–227	Ethyl acetate–cyclohexane	28	254 (16,600)	$C_{34}H_{27}N_3O_3$	77.69	5.18	8.00	77.25	5.21	7.88	>100
	Isomer B[d,i] 227–228	Ethyl acetate	50	253 (16,600)	$C_{34}H_{27}N_3O_3$	77.69	5.18	8.00	77.59	5.18	8.14	>100
XXIX[d]	220	Methanol	5	248 (15,500)	$C_{33}H_{24}BrN_3O_3$	67.12	4.10	7.12	67.05	4.50	7.06	>30 <100
XXX[j]	173–175	Ethyl acetate	8	248 (17,100)	$C_{35}H_{29}N_3O_3$	77.88	5.42	7.79	77.85	5.49	7.64	>10 <30
XXXI[k]	220 dec	Benzene	66	262 (22,200)	$C_{33}H_{25}N_3O_5$	70.11	4.20	7.73			7.89	>100

[a] Yields are of once-crystallized or precipitated products. Because of the many stereoisomers, isolations were tedious so that actual yields are probably higher than those shown. [b] Broad maxima which give multiple peaks at high concentration. [c] Approximate LD_{50} values based on 2/3 or 3/3 animals at dose levels of 10, 30, and 100 mg/kg. [d] Only *endo* isomers by nmr spectrum.[5] [e] Ethanol solvate by nmr. [f] Characterized by Michael J. Zelesko of these laboratories. [g] Contains approximately 30% *exo* isomers and 70% *endo* isomers by nmr spectrum.[5] [h] Amorphous powder. [i] Obtained from pure geometric fulvenylmethanol isomer.[4] Isomers A and B were different by mixture melting point determination, nmr spectra, and thin layer chromatography. [j] Contains approximately 70% *exo* isomers and 30% *endo* isomers by nmr spectrum.[5] [k] Contains approximately 40% *exo* isomers and 60% *endo* isomers by nmr spectrum.[5]

TABLE III

ISOMERS OF NORBORMIDE

Isomer	Stereochemistry[a]	% in mixture[b]	Toxicity (rat) LD_{50}, mg/kg *Po*	Toxicity (rat) LD_{50}, mg/kg Iv
Commercial mixture	...	...	5.3	0.65
"Y"	*trans,endo*	29	<5	0.50
"W"	*trans,endo*	16	*c*	5.0[d]
"V"	*cis,endo*	26	2.1	0.15
"U"	*cis,endo*	14	*c*	1.25–1.50
"X"	*cis,exo*	11	>100	>10
"R"	*cis,exo*	<1	*c*	>10
"T"	*trans,exo*	<2	*c*	>8.5
"S"	*trans,exo*	*e*	*c*	>8.0

[a] See ref 5. The bracketed isomers are *erythro–threo* pairs. Isomers "Y" and "V" are *threo* while isomers "W" and "U" are *erythro*. [b] Determined using a tlc method by Dr. C. Janicki of these laboratories. [c] Insufficient material. [d] The sample tested contained *ca.* 2% of isomer "Y." [e] Not detectable.

compound with an angular bromine atom (XXIX) was also weakly active.

Among the series of imide nitrogen substituted analogs, many were lethal to rats. Substitution by phenyl, cyclohexyl, or *t*-butyl gave compounds less than one-twentieth as toxic as II. Compounds with methylene groups attached to the imide nitrogen atom were active with the exception of N-benzylnorbormides. The potency of the N-methyl, -ethyl, -*n*-butyl, -2-methoxyethyl, and -2-pyridylmethyl compounds approached that of norbormide although none was more active. The N-*n*-propyl analog appears to be anomalous with an LD_{50} of 34.5 mg/kg, being considerably less active than the N-ethyl and N-*n*-butyl compounds.

Most striking is the effect of stereoisomerism on toxicity (Table III). The *exo* isomers "X," "R," "S," and "T"[5] failed to kill rats at doses 50–70 times the LD_{50} of the most active isomer. Most of the potency

of norbormide is due to the *endo* isomers "Y" and "V" which constitute about half of the commercial mixture and bear a *cis–trans* relationship to one another.[3] Isomerism at the carbinol carbon atom of norbormide has a very important effect on toxicity in that *endo* isomers "W" and "U," which bear an *erythro–threo* relation to isomers "Y" and "V,"[3] are no more than one-tenth as potent as isomers "Y" and "V."

It was not practical to carry out such a detailed stereochemical study with the various analogs of norbormide that have been prepared. Therefore, it has been assumed by analogy that substantial proportions of stereoisomers with "toxic configurations" were obtained. That a preponderance of *endo* isomers was present in the analogs of norbormide was shown by nmr spectra (see tables). These spectra also showed the presence of more than one isomer in most cases and could be roughly correlated with the spectra of the toxic norbormide isomers. Since unsymmetrical fulvenylmethanols analogous to I are obtained as a mixture of geometric isomers[4] and separation of *erythro–threo* pairs of norbormide isomers was very difficult,[3] it is reasonable to assume that mixtures of isomers of analogs are similar in composition to those obtained in the synthesis of norbormide.

Many other compounds related to norbormide in structure have been tested for raticidal effects, but found to be inactive. For example, the maleimide adduct of 6-phenyl-6-(2-pyridyl)fulvene,[2a,5] as well as related 7-diarylmethylene-5-norbornene-2,3-dicarboximides,[4] have failed to kill rats.

It may be concluded from the results of this limited study that the rat toxicity of norbormide is rather sensitive to structural changes and that activity of analogs of norbormide is only retained with the substitution of certain groups on the dicarboximide ring.

Experimental Section[6]

5-(α-Hydroxy-α-2-pyridylbenzyl)-N-methyl-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (III). Method A.—A solution of 9.5 g (0.023 mole) of α-phenyl-α-[6-phenyl-6-(2-pyridyl)-2-fulvenyl]-2-pyridinemethanol (I) (mixed isomers)[2,4] and 2.5 g (0.023 mole) of N-methylmaleimide[7] in 150 ml of benzene was heated under reflux for 1 hr and stirred at room temperature for 18 hr. The red color of I was discharged and a white crystalline product separated; 6.2 g (51%). Recrystallization from ethyl acetate gave III as white crystals: mp 209–211°; $\lambda_{max}^{CHCl_3}$ 2.90, 5.63, 5.88, 6.30, 6.80, 6.96 μ.

Using this procedure with I and the appropriate maleimides,[7] the following compounds (Table I) were prepared: IV, V, VI, VII, VIII, IX, X, XI, XIII, XVI, XVII, and XVIII. In some cases a longer heating period was necessary to complete the reaction. The extent of reaction was followed in many cases by measuring the ultraviolet absorption of unreacted fulvene.

Maleimide was caused to react with the corresponding fulvenylmethanols[4] by this method to give the ring analogs XXII, XXIII, XXV, XXVI, XXVII, and XXVIII of Table II. The fulvene precursor for XXIV has been described.[4]

2- (or 3-) Bromo-5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene-5-norbornene-2,3-dicarboximide (XXIX).—A solution of 4.14 g (0.01 mole) of I and 1.76 g (0.01 mole) of α-bromomaleimide[8] in 100 ml of benzene was heated under reflux. After 5 hr, the black solution showed only a 25% decrease in ultraviolet absorption at 325 mμ. After 24 hr heating, the mixture was cooled and filtered and the black sludge was triturated with 2-propanol to give a small amount of a tan solid. The solid was dissolved in chloroform and treated with Darco. After filtration, the $CHCl_3$ was removed *in vacuo* and the residue was recrystallized from methanol to give white crystals of XXIX: mp 220°; λ_{max}^{KBr} 2.95, 5.63, 5.80, 6.30, 6.70, 6.80, 6.90 μ.

5-(α-Hydroxy-α-2-pyridylbenzyl)-N,2- (or -N,3-) dimethyl-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (XXX).—A 4.14-g (0.01-mole) sample of I was combined with 1.25 g (0.01 mole) of freshly distilled α,N-dimethylmaleimide[3] in 25 ml of benzene and heated under reflux for 5 days. Periodic ultraviolet analysis at 325 mμ showed a gradual decline in the amount of I to 15% of the starting amount. The benzene was evaporated under vacuum and the residual oil was dissolved in ethyl acetate. Dilution with hexane and cooling returned 0.5 g (12%) of unchanged I. Evaporation of the filtrate gave an oil which could not be crystallized. One-half of this oil (2.3 g) was chromatographed over 75.0 g of neutral Woelm alumina no. 1 using benzene, ether, and ethanol. A 1.1-g fraction crystallized and, after recrystallization from ethyl acetate, provided 0.22 g (8%) of white crystalline XXX: mp 173–175°; λ_{max}^{KBr} 2.93, 5.65, 5.88, 6.30, 6.68, 6.90 μ. Thin layer chromatography on silica gel G (7:3 ethyl acetate–$CHCl_3$) showed a cluster of 3 spots.

N-[2-(N,N-Dimethylamino)ethyl]-5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (XV) (Method B).—Sodium ethoxide was prepared from 2.76 g (0.12 g-atom) of sodium and 200 ml of absolute ethanol. To this solution was added 25.5 g (0.05 mole) of II and the resultant slurry of sodium salt was heated under reflux with 10.1 g (0.07 mole) of 2-dimethylaminoethyl chloride hydrochloride for 9 hr (pH *ca.* 8). Sodium chloride was removed by filtration and the solvent was distilled *in vacuo*. The residue was dissolved in benzene, treated with Norit and the solution was concentrated to dryness. This crude gummy product showed no starting imide II by thin layer chromatography. Trituration with warm ether provided 17.4 g of ether-soluble material which very slowly crystallized from ether. Recrystallization from acetone–ether gave 6.93 g (23.6%) of XV, mp (128) 131–148°. This method was also used to prepare compounds III, XII, XIV, XIX, XX, and XXI of Table II.

The N-methyl compound III was also prepared from II and diazomethane in methanol in 100% yield.

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(5) Melting points are uncorrected and were determined on a Thomas-Hoover apparatus. Ultraviolet spectra were obtained with a Cary Model 14 spectrophotometer while infrared spectra were obtained with a Perkin-Elmer Model 21 spectrophotometer. The proton resonance spectra were run at 60 Mc in $CDCl_3$ on a Varian A60 spectrometer with a room temperature probe. The spectra of a few chloroform-insoluble compounds were run in dimethylformamide-*d*; or trifluoroacetic acid.

(7) N-Substituted maleimides were obtained from the Aero Chemical Corp., Newark, N. J.

(8) α-Bromomaleimide was obtained from maleimide with bromine by the method of R. A. Nicolaus and R. Nicoletti, *Rend. Accad. Sci. Fis. Mat.*, **26**, 149 (1959), in 60% yield, mp 155°.