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Stereochemical Properties and Teratogenic Activity of Some Tetrahydrophthalimides

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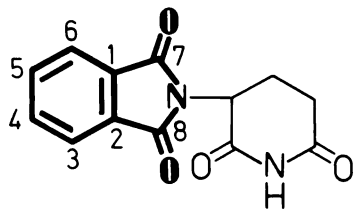
SUMMARY

FICKENTSCHER, K., KIRFEL, A., WILL, G. & KÖHLER, F. (1977) Stereochemical properties and teratogenic activity of some tetrahydrophthalimides. *Mol. Pharmacol.*, 13, 133-141.

The embryotoxic and teratogenic activities of three thalidomide analogues containing nonplanar tetrahydrophthalimide moieties, of the corresponding three imides, and of their *N*-methyl derivatives were tested in SWS mice. The molecular structures of three imides were established simultaneously by X-ray diffraction analysis. One of the three nonplanar thalidomide analogues is teratogenic and therefore does not support the intercalation hypothesis of the thalidomide action. The tendency of the three sulfur-containing compounds for complex formation is discussed.

INTRODUCTION

In spite of the efforts undertaken to find the cause of the teratogenic side effect of the hypnotic thalidomide (I) (1), the mechanism of this action is still unknown. Jönsson (2) has hypothesized that the flat phthalimide moiety[5] of I intercalates between adjacent base pairs of the DNA double helix. By "overlapping" of the aromatic phthalimide ring system with nucleic acid bases, so-called electron donor-acceptor complexes are formed. Within these, a nucleic acid base plays the role of an electron donor molecule, and the phthalimide portion acts as an electron acceptor because of the electron-withdrawing C=O groups of the carboxamide. Probably the nucleic acid base involved is a purine base (most likely guanine), since calculations show purine bases to be better electron donors than pyrimidine bases.



I

The intercalation hypothesis is unproved, but the total lack of teratogenicity in animal tests of some thalidomide analogues containing similar puckered phthalimide partial structures seems to confirm its validity. Two such nonteratogenic compounds are the hexahydro analogue II (6-9), the cyclohexane-1,2-dicarboximide portion of which is thought to be

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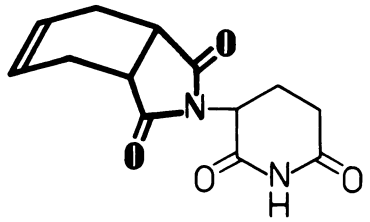
[5] Crystal structure analysis of I by X-ray diffraction has shown that the phthalimide plane and the main glutarimide plane are almost perpendicular (3-5).

in the boat form, and the somewhat bulkier compound III (10, 11), with a methylene bridge between C(3) and C(6) (see stereoformulae of II and III).

In terms of Jönsson's theory intercalation ought to be prevented in both compounds by steric hindrance. If the intercalation principle of thalidomide action is correct, neither 3,4,5,6-tetrahydrothalidomide (IV), its 3,6-dithia analogue (VII), nor the 1,2,3,6-tetrahydro compound (X) should give rise to teratogenic activity, since all three compounds possess nonplanar tetrahydrophthalimide moieties. This applies especially to X, with an expected configuration similar to II.

So far the role of the α-glutarimide residue in the teratogenic effect of I is not clear. Although, according to Jönsson's detailed hypothesis, the glutarimide ring is of fundamental importance for the embryopathic effect of I, other investigations (8, 12) have suggested that this portion of the molecule may influence the degree of activity but is not primarily important for initiating this effect. Therefore, in addition to the three thalidomide analogues, IV, VII, and X, their corresponding unsubstituted tetrahydrophthalimides, VI, IX, and XII, and also their *N*-methyl derivatives, V, VIII, and XI, have been prepared and tested for teratogenic and embryotoxic activities in animals.



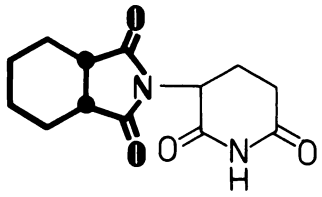
Stereoformula of X

In order to gain additional information about structure-activity relationships, the molecular configurations of VI, IX, and XII have been established by X-ray diffraction analysis.

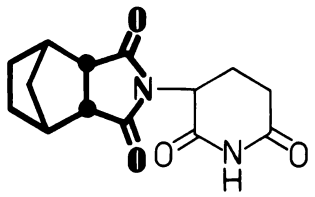
METHODS AND RESULTS

The compounds were synthesized as described in the APPENDIX.

Crystal structure analysis. Single crystals of compounds XI, IX, and XII, about 0.2 × 0.2 × 0.2 mm, were investigated on an automatic four-circle diffractometer using molybdenum radiation. The structures were solved by use of the direct-method computer program MULTAN (13) and refined by least-squares calculations. Details of the X-ray techniques have been



II



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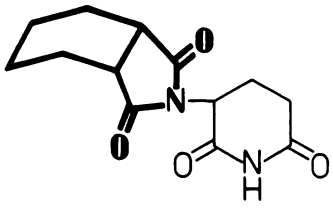
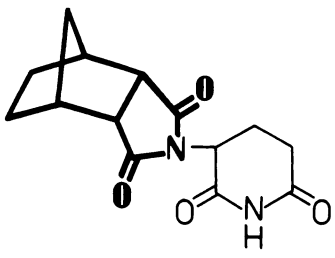
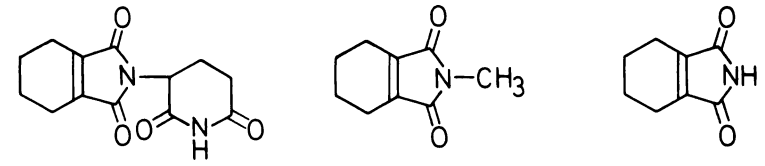


Stereoformula of II



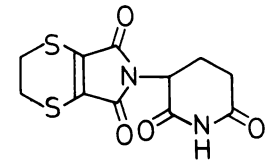
Stereoformula of III



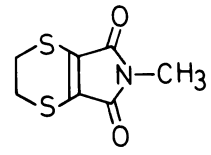
IV

V

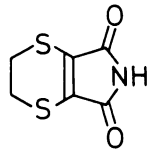
VI



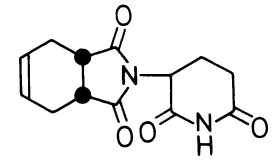
VII



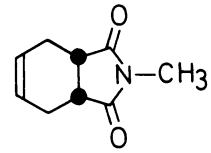
VIII



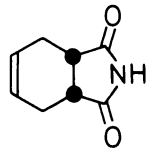
IX



X



XI



XII

described previously (14–16).

Figures 1, 2, and 3 show ORTEP drawings (17) of the molecules VI, IX, and XII, respectively. As expected, in both VI and IX the 5-membered imide rings and those parts of the 6-membered rings defined by atoms 1, 2, 3, and 6 are essentially planar. The valences C(3)–C(4) and C(6)–C(5) in VI, as well as S(3)–C(4) and S(6)–C(5) in IX, are oriented in opposite directions to the planes, thus causing the 6-membered rings to be generally nonplanar. In both molecules the deviations of C(4) and C(5) from the plane are not equal, as expected from the chemical symmetry of the molecules. In VI the deviations are 0.345 A for C(4) and 0.407 A for C(5). In the 3,6-dithia analogue IX the corresponding values are 0.518 A and 0.300 A. Since the molecular chemistry presents no obvious reason for these different deviations, they must be due to intermolecular forces in the crystals. For the molecules in solution the opposite deviations can be estimated from the mean deviations of 0.376 A for VI and 0.409 A for IX. In the latter compound the mean deviation of C(4) and C(5) is larger because of the more acute bond angles around the sulfur atoms in positions 3 and 6. The bulkiest parts of the molecules are determined by the C(4)–H and C(5)–H valences, which are oriented in opposite directions almost perpendicular to the molecular plane. Taking the van der Waals radius of hydrogen as 0.5 A, the thickness of both molecules at positions 4 and 5 of the 6-membered ring is about 5 A. Hence there is a considerable deviation from flatness, and it seems doubtful that intercalation of the molecules from the 6-membered ring side is possible. Compound IX is a slightly thicker molecule than VI, but apart from this minor difference, both molecules may be regarded as stereochemically equivalent.

In XII (Fig. 3) the assumed tilted configuration of the molecule can be confirmed. The 6-membered ring is in a boat form and folds toward the planar imide ring. The plane defined by the atoms of the 6-membered ring forms an angle of 108° with the imide ring, and this configuration implies that steric hindrance of the 6-membered ring should prevent intercalation or at least confine it to the outermost region of the 5-membered ring.

Animal experiments. The animal experiments were performed with mice of the

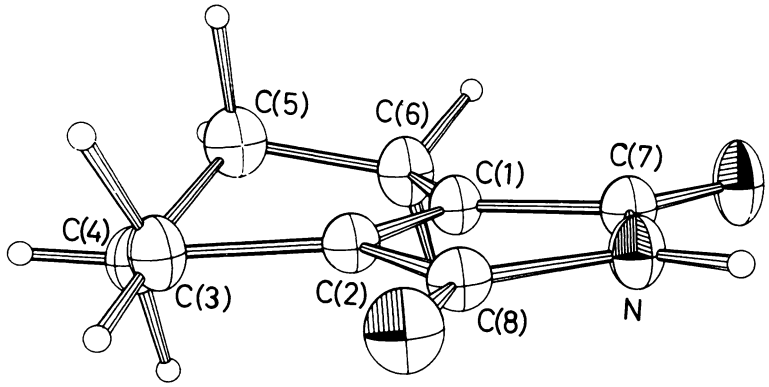


FIG. 1. *Molecular structure of compound VI*

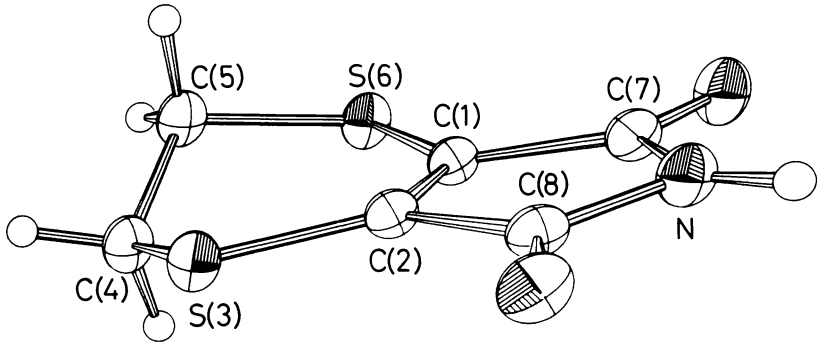


FIG. 2. *Molecular structure of compound IX*

inbred line SWS 53/65. The substances were administered on day 9 of gestation by a single intraperitoneal injection, usually given to 5 or 10 pregnant females for each dose stage (see Table 1). The drugs were dissolved in a 3:1 (v/v) mixture of 0.9% NaCl and Tween 20, and the injected volume was 10 ml/kg. Control series 1 was given medium alone (40 ml/kg); control series 2 was untreated. On day 18 of gestation the animals were killed with ether, and the fetuses were removed and macerated in potassium hydroxide solution. The skeletons were stained with alizarin red (18) and inspected for anomalies.

The results of the animal tests are summarized in Table 1. The toxic effect on the mothers is expressed as the lethal dose for 50% of the animals (LD_{50}). The embryotoxic effect is measured both by the resorption rate, R/I, and by the embryolethal dose for 50% of the fetuses, ELD_{50} (the dose giving a 50% resorption rate). Teratogenic activity is expressed by the malformation rate, M/F, and the teratogenic dose for 50% of the fetuses, TD_{50} (the dose giving a 50% malformation rate). The observed skeletal anomalies were the same as described previously (11, 19): thumb or radial reductions, melted or forked thoracic vertebrae or ribs, radial and tibial aplasia (sometimes extending to the first or second phalanx), or complete amelia, and severe phocomelia of the long bones.

In order to compare maternal with embryonal toxicities, the compounds were ranked in descending order according to their interpolated LD_{50}, ELD_{50}, and TD_{50} values (millimoles per kilogram). For maternal toxicity (LD_{50}): VIII (0.10) > IX (0.27) > XII (1.24) > V (1.36) > XI (2.12) > X (2.67). For embryolethality (ELD_{50}): VI (0.04) > VIII (0.13) ≈ IV (0.14) > IX (0.33) > V (0.39) ≈ VII (0.40) > XII (0.53) > I (1.18) > XI (1.66) > X (2.34). For teratogenicity (TD_{50}): VI (0.06) ≈ VIII (0.07) > IX (0.37) ≈ XII (0.39) > V (0.48) > VII (0.64) > I (0.83) > XI (1.42).

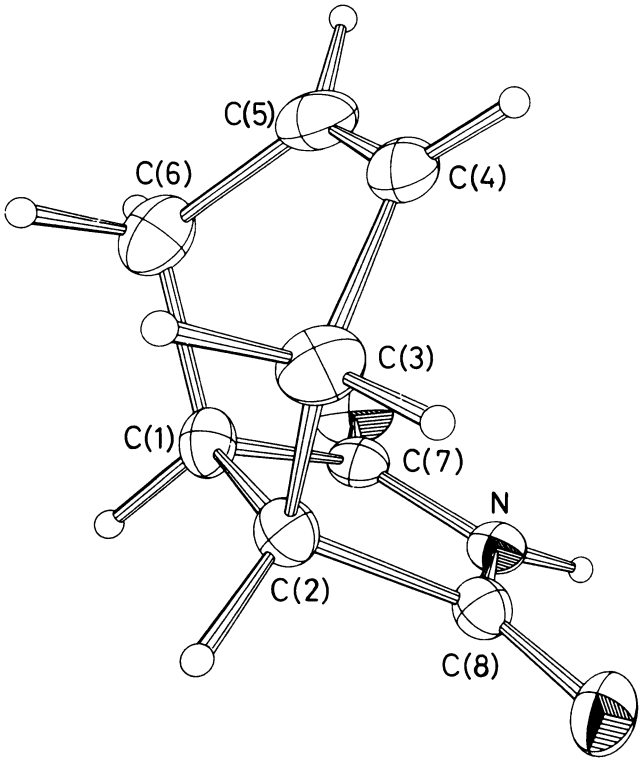


FIG. 3. *Molecular structure of compound XII*

DISCUSSION

The relatively low teratogenicity of the reference compound I, with a TD_{50} of 0.83 mmole/kg, can be explained partially by its rapid, spontaneous hydrolysis at physiological pH values to yield all 12 of the theoretically possible metabolites (20). None of the polar compounds were found to be active in animal tests (8). Since the results of teratological tests performed with I are not easily reproducible because of the rapid breakdown of the molecule, it seems to be an unsuitable model compound for teratological studies.

For the two thalidomide analogues IV and X, no teratogenic effects could be found up to the tolerance limits of 0.19 and 1.52 mmoles/kg, respectively. Replacement of the puckered glutarimide residue in IV and X with a methyl group (V and XI) or with a hydrogen atom (VI and XII) produced teratogenicity. Likewise, the effect of VII (TD_{50} = 0.64 mmole/kg) was increased about 10-fold by replacement of the glutarimide residue with a methyl group (VIII; TD_{50} = 0.07 mmole/kg). Similarly, phthalimide (TD_{50} = 0.09 mmole/kg) has 10 times the activity of its α-glutarimide derivative I, as recent investigations (12) have shown. So far these results suggest that the bulky glutarimide portion generally decreases teratogenic potency.

The nonplanar compounds V–IX, XI, and XII all show teratogenic effects, with VI and VIII being the most active, as expressed by their low TD_{50} values. This finding seems in conflict with the intercalation hypothesis, especially for the almost spherical compound XII (TD_{50} = 0.39 mmole/kg), which is thought to be unable to intercalate at all. Agreement of these results with the intercalation concept seems possible only if partial intrusion of the glutarimide-free molecules is assumed, with the flat regions of their 5-membered imide rings between adjacent base pairs of DNA locally extending and untwisting the double helix. The activity of the glutarimide derivative VII remains puzzling.

TABLE 1

Toxicity, embryotoxicity, and teratogenicity of compounds I and IV–XII in SWS mice

Compound	Dose		Maternal data			Embryonal data					
			Pg[a]	Ab	Lt	I	R	F	M	R/I	M/F
	mg/kg	*mmoles/kg*								%	%
I	3	0.012	10	0	0	131	5	126	0	3.8	0
	6	0.023	10	0	0	127	9	118	4	7.1	3.4
	12	0.046	10	0	0	133	15	118	2	11.3	1.7
	25	0.097	14	0	0	179	31	148	19	17.3	12.8
	50	0.194	11	0	0	141	28	113	17	19.9	15.0
	100	0.387	10	0	0	125	30	95	26	24.0	27.4
	200	0.775	12	0	0	153	65	88	41	42.5	46.6
	400	1.549	16	0	0	203	108	95	82	53.2	86.3
IV	12.5	0.048	5	0	0	60	4	56	0	6.7	0
	25	0.095	5	0	0	53	17	36	0	32.1	0
	50	0.191	5	1	0	41	31	10	0	75.6	0
	100	0.381	5	4	0	12	12	0	0	100	0
V	6.25	0.038	10	0	0						
	12.5	0.076	10	0	0	125	10	115	0	8.0	0
	25	0.151	10	0	0	131	13	118	0	9.9	0
	50	0.303	10	2	0	127	26	101	8	20.5	7.9
	100	0.605	10	1	2	107	43	64	15	40.2	23.4
	200	1.211	10	5	5	91	68	23	23	74.7	100
VI	1.56	0.010	5	0	0	55	1	54	0	1.8	0
	3.12	0.021	5	0	0	55	15	40	0	27.3	0
	6.25	0.041	5	0	0	60	38	22	2	63.3	9.1
	12.5	0.083	5	0	0	67	60	7	5	89.6	71.4
	25	0.165	5	1	0	39	39	0	0	100	0
VII	5	0.017	5	0	0	59	5	54	0	8.5	0
	10	0.034	5	0	0	60	6	54	0	10.8	0
	20	0.067	5	0	0	59	6	53	0	10.2	0
	40	0.134	5	0	0	58	16	42	1	27.6	2.4
	80	0.268	5	0	0	57	19	38	8	33.3	21.1
VIII	1.56	0.008	5	0	0	68	4	64	0	5.9	0
	3.12	0.016	5	0	0	65	9	56	0	13.9	0
	6.25	0.031	5	1	0	53	10	43	4	18.9	9.3
	12.5	0.062	5	2	0	51	15	36	14	29.4	38.9
	25	0.124	5	1	2	37	18	19	19	48.7	100
	50	0.249	5	1	4						
IX	25	0.134	5	0	0	52	19	33	0	36.5	0
	50	0.268	5	2	0	37	15	22	8	40.5	36.4
	100	0.534	5	0	5						
X	50	0.190	5	0	0	63	3	60	0	4.8	0
	100	0.381	5	0	0	66	3	63	0	4.5	0
	200	0.762	5	0	0	61	10	51	0	16.4	0
	400	1.524	5	1	1	40	13	27	0	32.5	0
	800	3.049	5	0	5						
XI	25	0.151	10	0	0	133	7	126	0	5.3	0
	50	0.302	10	0	0	127	9	118	0	7.1	0
	100	0.605	10	2	1	93	10	83	0	10.8	0
	200	1.211	10	3	2	63	23	40	17	36.5	42.5
	400	2.421	10	0	10						

TABLE 1—*Continued*

Compound	Dose		Maternal data			Embryonal data					
			Pg[a]	Ab	Lt	I	R	F	M	R/I	M/F
	mg/kg	*mmoles/kg*								%	%
XII	6.25	0.041	10	0	0	141	8	133	0	5.7	0
	12.5	0.083	10	0	0	134	13	121	5	9.7	4.1
	25	0.165	10	0	0	137	19	118	11	13.9	9.3
	50	0.331	10	1	0	119	24	95	19	20.2	20.0
	100	0.661	10	4	2	53	33	20	17	62.3	85.0
	200	1.323	10	2	8						
Control series 1	40 ml/kg		10	0	0	135	8	127	0	5.9	0
Control series 2	0		10	0	0	127	9	118	0	7.1	0

Compound	Maternal toxicity: LD_{50}		Embryonal toxicity			
			ELD_{50}		TD_{50}	
	mg/kg	*mmoles/kg*	*mg/kg*	*mmoles/kg*	*mg/kg*	*mmoles/kg*
I			305.7	1.18	214.7	0.83
IV			35.7	0.14		
V	224.9	1.36	64.7	0.39	78.4	0.48
VI			5.3	0.04	8.8	0.06
VII			120.0	0.40	189.6	0.64
VIII	31.3	0.16	25.6	0.13	14.3	0.07
IX	49.8	0.27	61.8	0.33	68.7	0.37
X	700.3	2.67	615.5	2.34		
XI	350.3	2.12	274.1	1.66	235.2	1.42
XII	187.5	1.24	80.0	0.53	58.8	0.39

[a] The abbreviations used are: Pg, pregnant; Ab, abortion within 0–5 days after administration; Lt, lethal within 0–5 days after administration; I, implantations; R, resorptions; F, fetuses; M, malformed fetuses; R/I, resorption rate; M/F, malformation rate; ELD_{50}, lethal dose for 50% of the fetuses (i.e., dose giving a 50% resorption rate); TD_{50}, teratogenic dose for 50% of the fetuses (i.e., dose giving a 50% malformation rate).

While replacement of the methyl group in V and XI with a hydrogen atom (VI and XII) leads to an increase in teratogenic potency, the free imide IX (TD_{50} = 0.37 mmole/kg) is only one-fifth as active as the corresponding methyl derivative VIII (TD_{50} = 0.07 mmole/kg). This surprising difference is probably due to the relatively high acidity of IX, which might lead to difficulty in penetrating the cell membrane.[6] The higher acidity of IX in comparison with its stereochemically equivalent analogue VI is based on the special quasi-aromatic feature of the planar partial 6-membered ring region limited by C(1)–C(2)–S(3)–S(6). Each sulfur atom provides 2 of its 4 3*p* electrons, and together with the two π-electrons of the C(1)=C(2) double bond, a sort of aromatic system is formed (22–24). This structural feature imparts to compounds VII, VIII, and IX chemical reactivities—including a tendency toward complex formation—similar to the corresponding phthalimides (22–24). In this way both the teratogenic activity of the glutarimide compound VII and the extremely potent effect of VIII may be explained.

The most important conclusion to be drawn from the present results is that compounds of the thalidomide type, with nonplanar molecular structures, are teratogenic. This must cast considerable doubt on the intercalation hypothesis, the valid-

[6] Comparable results were obtained in corresponding investigations on dichloromaleinimides (21).

ity of which must await further experimentation.

APPENDIX

α-Phthalimidoglutarimide (thalidomide). (I). Compound I (m.p. 271°) was obtained from Firma Chemie Grünenthal, Stolberg.

α-(3, 4, 5, 6-Tetrahydrophthalimido)-glutarimide (IV). Compound IV (m.p. 207°) was prepared according to Fickentscher (25).

3, 4, 5, 6-Tetrahydrophthalimide (VI). Compound VI (m.p. 171°) was prepared according to Volkov and Shugal (26).

N-Methyl-3, 4, 5, 6-tetrahydrophthalimide (V). To a solution of 2.0 g (13.2 mmoles) of VI in 20 ml of dry acetone, 1.82 g (13.2 mmoles) of K_2CO_3 and 1.7 g (13.2 mmoles) of freshly distilled dimethyl sulfate were added. The mixture was refluxed for 10 hr to yield 2.1 g (96%) of V, m.p. 51° (from methanol–water).

$C_9H_{11}NO_2$

Calculated: C 65.44, H 6.71, N 8.48
Found: C 65.38, H 6.76, N 8.29

α-(3, 6-Dithia-3, 4, 5, 6-tetrahydrophthalimido) glutarimide (VII). Compound VII (m.p. 269°) was prepared according to Fickentscher (22).

3,6-Dithia-3,4,5,6-tetrahydrophthalimide (IX). Compound IX (m.p. 216°) was prepared according to Schweizer (24).

N-Methyl-3, 6-dithia-3, 4, 5, 6-tetrahydrophthalimide (VIII). Compound VIII was prepared by analogy with V, with 5 g (27 mmoles) of IX in 60 ml of dry acetone, 3.73 g (27 mmoles) of K_2CO_3, and 3.4 g (27 mmoles) of freshly distilled dimethyl sulfate. Refluxing for 2 hr yielded 5.5 g (97%) of VIII, m.p. 173° (from glacial acetic acid–water).

$C_7H_7NO_2S_2$

Calculated: C 41.80, H 3.51, N 6.96
Found: C 41.59, H 3.65, N 7.14

α-(1,2,3,6-Tetrahydrophthalimido) glutarimide (X). Compound X (m.p. 195°) was prepared according to Koch and Kotlan (9).

1,2,3,6-Tetrahydrophthalimide (XII). Compound XII (m.p. 139°) was purchased from Firma E. Merck, Darmstadt.

N-Methyl-1,2,3,6-tetrahydrophthalimide (XI). Compound XI was prepared by analogy with V, with 2.0 g (13.2 mmoles) of XII in 20 ml of dry acetone, 1.82 g (13.2 mmoles) of K_2CO_3, and 1.7 g (13.2 mmoles) of freshly distilled dimethyl sulfate and refluxing for 2 hr. After cooling to room temperature, the mixture was weakly acidified with concentrated HCl. The oily precipitate crystallized at 0° to give 1.6 g (74.5%) of XI, m.p. 68° (from methanol–water).

$C_9H_{11}NO_2$

Calculated: C 65.44, H 6.71, N 8.48
Found: C 65.61, H 6.83, N 8.63

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