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A Study of the Inhibitory Effect of Various Hydrazides on Monoamine Oxidase *in vitro* and *in vivo*

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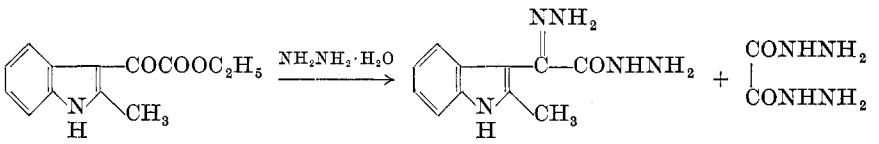
Introduction

The work described in this paper was initiated by an observation that a by-product from a somewhat unusual chemical reaction showed some unexpected pharmacological activity.

During an attempted preparation of 2-methyl-3-indoleglyoxylic acid hydrazide, ethyl 2-methyl-3-indoleglyoxylate (I)* was subjected to hydrazinolysis with hydrazine hydrate.



(I) (II) (1)

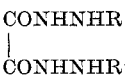
This reaction afforded the hydrazone-hydrazide (II) and oxalic acid bishydrazide (1).* Compound 1 was active as an inhibitor of tryptophan decarboxylase *in vitro* ($[I]_{50}$ $5 \cdot 9 \times 10^{-6}$)†, but was orally inactive in mice. This observation prompted us to prepare a series of related oxalic acid bishydrazides (see Table I) and also various other aliphatic hydrazides (see Table II) and aromatic hydrazides (see Table III). Since we were primarily interested in the inhibition of the monoamine oxidase system,‡ these compounds were studied particularly with respect to their activity toward this enzyme.

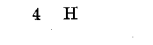
* Roman numerals refer to compounds mentioned only in the text, while Arabic numerals refer to compounds which are also included in Table I.

† $[I]_{50}$ is the molar concentration of the compound causing a 50 per cent inhibition of the enzyme under the conditions of the experiment.

‡ Referred to frequently as MAO.

Table I. Oxalic acid bishydrazides



Cmpd. no.	R	R′	Ultraviolet spectrum[k], mμ		Infrared spectrum, cm^{-1}		
			Neutral in H_2O	In base	NH/OH	C=O	Amide II
1	H	H[a]	218 (6,750)		3260 3140	1680	1538
2	H	$CH(CH_3)_2$[b]	238 (6,400)	240 (7,600) f272 (5,750)	3230	1697w 1656 1622	1519
3	H	$CH_2CH_2C_6H_5$[b]	236 (6,750)	238 (7,950) sh268 (6,900)	3270 3220 3100	1686 1660	1548sh 1535
4	H		236 (6,600)	260 (6,150)	3280 3200 3150	1675sh 1665	1555 1540
5	CH_3	CH_3[c]	232 (5,200)	272·5 (5,900)	3220	1653	1563
6	C_2H_5	C_2H_5[b]	237 (5,550)		3240	1660 1641	1533
7	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$[b]	238 (5,450)	279 (6,900)	3240	1640	1533
8	$CH(CH_3)_2$	$CH(CH_3)_2$[b]	238 (5,700)	220 (4,750) 274 (6,550)	3230 3180	1650	1540
9	C_2H_5	$CH(CH_3)_2$[b]	237 (5,550)	274 (6,350)	3240 3200	1650	1545
10	n-C_4H_9	n-C_4H_9[b]	236 (5,400)		3200 3070	1690 1660	1555
11			242 (5,800)		3280sh 3240 3180	1673 1655	1534
12			240 (5,600)		3240 3180	1675 1665	1550sh 1537
13			244 (5,850)		3280	1655	1505
14	$CH_2C_6H_5$	$CH_2C_6H_5$[b]	f236 (6,350)	277 (7,300)	3190 3130	1670 1655	1565
15	$CH_2CH_2C_6H_5$	$CH_2CH_2C_6H_5$[b]	238 (6,000)		3220 3200sh	1655sh 1640 1630	1538
16			plateau 236 (6,200)	275 (7,650)	3260	1642	1545
17	$CH(CH_3)_2$		236 (5,200) f256 (4,550)		3270 3230sh	1642	1548sh 1540
18	isomer I 		236 (5,900)	f226 (9,700) 276 (6,650)	3300sh 3240	1673 1655 1630	1520

Formula	Analyses, %								LD_{50},[f] mg/kg	Monoamine oxidase inhib., %	
	Calcd.				Found					at 10^{-3}M	$[I]_{50}$
	C	H	N	N.E.[i]	C	H	N	N.E.[i]			
									200	0	
$C_6H_{12}N_4O_2$	37·49	7·55	34·98	80·09	37·57	7·73	35·35	83	65		$1·5\times10^{-4}$
$C_{10}H_{14}N_4O_2$	54·04	6·35	25·41	—	54·47	6·41	25·46	—	767		5×10^{-5}
$C_{11}H_{16}N_4O_2$	55·91	6·83	23·72	118·1	56·09	6·91	24·06	118	200		$1·3\times10^{-4}$
									1000	18	
$C_6H_{14}N_4O_2$	41·37	8·10	32·17	87·1	41·50	8·16	32·43	87·6	300		5×10^{-5}
$C_6H_{18}N_4O_2$	47·50	8·97	27·70	101·13	47·52	9·26	27·32	99·5	650		$5·2\times10^{-4}$[h]
$C_6H_{18}N_4O_2$	47·50	8·97	27·20	101·13	47·27	9·07	27·77	101	650		$5·7\times10^{-5}$
$C_7H_{16}N_4O_2$	44·66	8·57	29·77	—	44·26	8·54	29·68	—	167		$2·1\times10^{-4}$
$C_{10}H_{22}N_4O_2$	52·15	9·63	24·33	—	52·11	9·49	24·35	—	533		$3·1\times10^{-4}$[h]
$C_{10}H_{22}N_4O_2$	52·15	9·63	24·33	—	52·36	9·50	24·42	—	650		$3·6\times10^{-4}$[h]
$C_{10}H_{22}N_4O_2$	52·15	9·63	24·33		52·66	9·69	24·43		533		$1·8\times10^{-4}$
$C_{12}H_{26}N_4O_2$	55·78	10·14	21·69		55·77	10·09	21·71		767		$2·8\times10^{-4}$[h]
$C_{16}H_{18}N_4O_2$	64·41	6·08	18·78		64·40	6·25	19·29		650		$2\cdot10^{-4}$
$C_{18}H_{22}N_4O_2$	66·23	6·79	17·17		66·16	6·89	17·90		>1000		3×10^{-5}[h]
$C_{20}H_{26}N_4O_2$	67·77	7·39	15·81		67·89	7·48	15·85		538		$1·8\times10^{-4}$[h]
$C_{14}H_{22}N_4O_2$	60·41	7·97	20·13		60·85	8·15	19·91		533		$8·0\times10^{-5}$
$C_{12}H_{22}N_4O_2$	56·67	8·72	22·03		56·75	8·79	22·33		650	0	

Table I—*continued*

Cmpd. no.	R	R′	Ultraviolet spectrum[k], mμ		Infrared spectrum, cm^{-1}		
			Neutral in H_2O	In base	NH/OH	C=O	Amide II
19	CH_3 | CH—⟨ isomer II	CH_3 | CH—⟨ [b]	235 (5,000)	226 (4,750) 276 (6,600)	3220	1640	1548
20	CH_3[d] and CH_3	CH_3 and CH_3[e]	f244 (3,000)		3200	1660	1525
21	⟨	⟨ [b]	f228 (5,450) 240 (5,800)	276 (6,900)	3240 3200	1677 1655	1535 1525
22	C_6H_5[d]	C_6H_5[b, f]	dimethyl-formamide-ethanol 279 (5,400) f324 (1,100)		3270 3110 3020	1690 1693	1533
23	CH_3[d] and C_6H_5	CH_3 and C_6H_5[g]	239 (27,700) 283 (5,000) f324 (1,150)		3210 3170	1683 1665	1520
24	$CH(CH_3)_2$	CH_2OH | $CH—CH_2OH$[b]	238 (5,550)	276 (6,550)	3350 3130 3070sh	1635	1565sh 1548
25	CH_2OH | $CH—CH_2OH$	CH_2OH | $CH—CH_2OH$[b]	238 (5,100)		3300 3170	1675	1540
26	$COCH_3$[d] and $CH(CH_2OCOCH_3)_2$	$COCH_3$ and $CH(CH_2OCOCH_3)_2$[b]	sh288 (1,400)		3250	1740 1725 1702 1687	1500
27	CH_3 | $CH—CHOH—CH_3$	CH_3 | $CH—CHOH—CH_3$[b]	242 (5,350)	278 (6,700)	3430 3260	1688 1655	1542
28	H	$CH_2CH_2CONHCH_2—$[b]	243 (6,950)	244 (6,850) f272 (5,800)	3210 3120 3080	1652 1620	1570
29	$CH_2CH_2COHNCH_2C_6H_5$	$CH_2CH_2CONHCH_2C_6H_5$[b]	236 (5,500) f256 (4,650)	274 (6,300)	3300 3290 3180	1633	1560

[a] Prepared in quantitative yield according to Borsche *et al.*[1]

[b] For synthesis see the Experimental section.

[c] Brüning;[2] m.p. 221–221·5°. Michaelis and Hadanck,[3] showed that the compound does not form a benzylidene derivative.

[d] These compounds do not fit the generic formula above since they are 2,2′-tetrasubstituted.

[e] Prepared according to Renouf,[4] m.p. 200°.

[f] Prepared previously from oxalyl chloride and phenylhydrazine by Folpmers[5]; m.p. 277°. Our sample was made from diethyl oxalate and phenylhydrazine and was crystallized from pyridine.

[g] Prepared previously by Folpmers[6]; m.p. 196–197° from *N*-methyl-*N*-phenylhydrazine hydrochloride and oxalyl chloride. Our sample was made from diethyl oxalate and *N*-methyl-*N*-phenylhydrazine and was crystallized from ethanol.

[h] Run in suspension.

[i] Neutral equivalent was determined in acetic acid–perchloric acid.

[j] LD_{50} value was determined i.p. in mice.

[k] 'Neutral' refers to 95 per cent ethanol unless otherwise specified. 'Base' refers to 0·01N KOH.

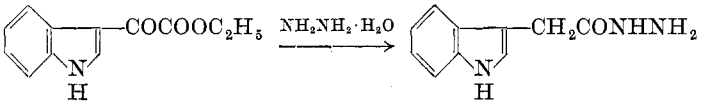
Formula	Analyses, %								LD_{50},[j] mg/kg	Monoamine oxidase inhib., %	
	Calcd.				Found					at 10^{-3}M	$[I]_{50}$
	C	H	N	N.E.[i]	C	H	N	N.E.[i]			
$C_{12}H_{22}N_4O_2$	56·67	8·72	22·03		56·67	8·85	22·17		650		$2·7\times10^{-4}$
$C_6H_{14}N_4O_2$	41·37	8·10	32·17		41·54	8·01	31·66		>10000	0	
$C_{12}H_{22}N_4O_2$	56·67	8·72	22·03		57·04	8·65	22·25		767	54[h]	
$C_{14}H_{14}N_4O_2$	62·21	5·22	20·73		62·11	5·64	20·52		>1000	0[h]	
$C_{16}H_{18}N_4O_2$	64·41	6·08	18·78		64·52	6·28	18·81		650	0[h]	
$C_8H_{18}N_4O_4$	41·01	7·75	23·92		40·74	7·98	24·27		>1000		$2·4\times10^{-5}$
$C_8H_{18}N_4O_5$	36·09	6·81	21·04		36·33	7·02	21·25		>1000		4×10^{-5}
$C_{20}H_{30}N_4O_{12}$	46·33	5·83	10·81		46·89	6·15	11·26		>1000	0	
$C_{10}H_{22}N_4O_4$	45·79	8·45	21·36		45·93	8·75	21·73		>1000	56	
$C_{12}H_{17}N_5O_3$	51·60	6·14	25·08		51·29	5·95	24·92		650		$3·4\times10^{-5}$[h]
$C_{22}H_{28}N_6O_4$	59·98	6·41	19·08		59·76	6·60	19·13		650		$2·0\times10^{-4}$

Table II. Various aliphatic hydrazides

Compound	Ultraviolet spectrum, mμ		Infrared spectrum, cm^{-1}				Analyses, %						LD_{50}, mg/kg	Monoamine oxidase inhibit., % at 10^{-3} M	$[I]_{50}$
							Calcd.			Found					
	Neutral	In base	NH/OH	C=O	Amide II	Formula	C	H	N	C	H	N			
$HCONHNH_2$[a]													65	0	
$CH_3CONHNH_2$[b]	end absorption		3270 3230 3010	1657 1625	1533								166	0	
$CH_3CONHNHCH(CH_3)_2$[b]	end absorption		3240	1640	1545	$C_5H_{12}N_2O$	51·70	10·41	24·12	51·56	10·51	24·11	300	46	
$CH_3CH(CH_3)CONHNH_2$	f228 (1,050)		3270 3160	1635	1535								767	0	
$CH_3CH(CH_3)CONHNHCH(CH_3)_2$[d]	f236 (446)		3290 3240	1644	1545	$C_7H_{16}N_2O$	58·30	11·18	19·43	58·07	11·14	19·36	253	44	
$CONHNH_2$ | $CH_2CONHNH_2$[e]	end absorption	228 (5,650)	3260 3110 3000	1675 1643	1535								>1000	16	
$CO_2NHNHCH(CH_3)_2$ | $CH_2CONHNHCH(CH_3)_2$[c]	end absorption		3320 3240	1665 1641	1559 1529	$C_9H_{20}N_4O_2$	49·98	9·32	25·91	50·01	9·11	25·73	>1000	0	
$CH_2CONHNH_2$[f] | $CH_2CONHNH_2$	end absorption		3280 3160 3020	1631	1537								>1000	14	
$CH_2CONHNHCH(CH_3)_2$[c] | $CH_2CONHNHCH(CH_3)_2$	end absorption	f214 (7,800)	3280	1640	1540 1505	$C_{10}H_{22}N_4O_2$	52·15	9·63	24·33	52·14	9·46	24·46	>1000		$1·8\times10^{-4}$
$CO(NHNH_2)_2$[g]	227 (4,150)		3340 3280 3190	1645	1550 1540								167	15	
$CONHNHCH(CH_3)_2$[c] | $NHNH(CH_3)_2$	end absorption		3260 3140 3070	1663	1540	$C_7H_{18}N_4O$	48·25	10·41	32·16	48·08	10·06	31·94	200	0	

[a] Prepared according to Pellizzari;[7] m.p. 54°, hygroscopic. [b] Prepared according to Curtius and Hoffman;[8] m.p. 67°, hygroscopic. [c] For synthesis see Experimental section. [d] Prepared according to Stollé and Gutmann;[9] m.p. 104°. [e] Prepared according to Bülow and Weidlich;[10] m.p. 154°. [f] Prepared according to Bülow and Weidlich;[11] m.p. 166°. [g] Prepared according to Mohr *et al.*;[12] m.p. 153–154°.

As an extension of the above reaction, ethyl 3-indoleglyoxylate (III) was subjected to condensation with hydrazine hydrate, and the hydrazide of indole-3-acetic acid (IV) was isolated in good yield.



(III) (IV)

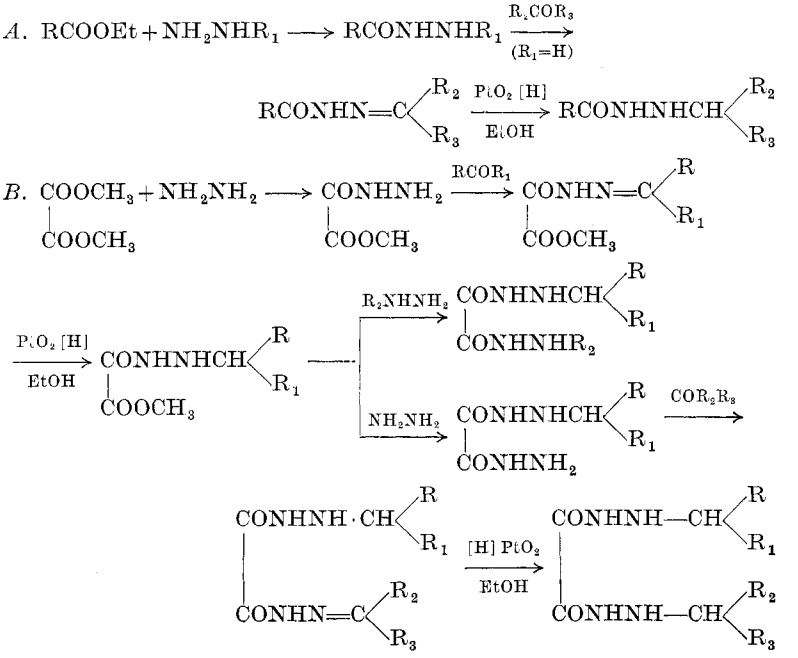
A similar Huang–Minlon type of reduction, which takes place under mild conditions due to the activation by an adjacent carbonyl and does not require potassium hydroxide, is reported in the case of an 11,12-diketosteroid.[17]*

The reaction of 3-indoleglyoxylyl chloride with hydrazine gave rise to 3-indoleglyoxylic acid hydrazide and 1,2-bis(3-indoleglyoxylyl)-hydrazine.

Discussion

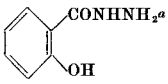
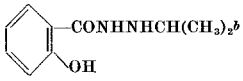
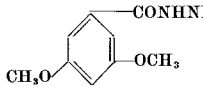
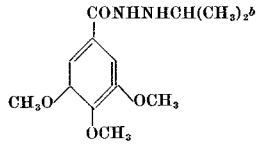
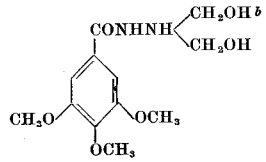
Syntheses

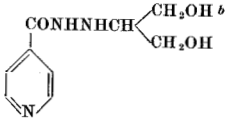
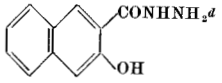
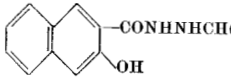
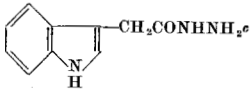
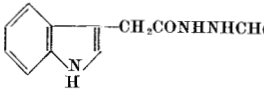
The hydrazides compiled in Tables I, II and III were synthesized by means of one of the following methods:



* After the completion of this manuscript Nenitzescu and Răileanu[18] have described the preparation of indole-3-acetic acid from indole-3-glyoxylic acid and hydrazine hydrate in the presence of potassium hydroxide.

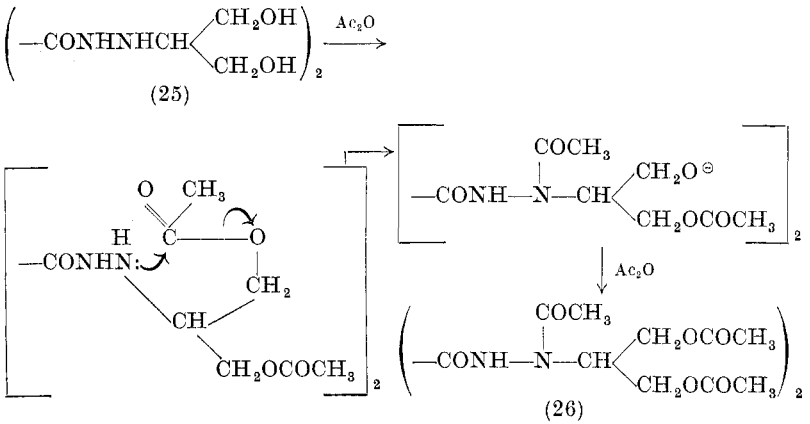
Table III. Aromatic hydrazides

Compound	Ultraviolet spectrum, mμ		Infrared spectrum, cm⁻¹			Formula	Analyses, %						LD_{50}	Monoamine oxidase inhibition, % at 10^{-3} M $[I]_{50}$
							Calcd.			Found				
	Neutral	In base	NH/OH	C=O	Amide II		C	H	N	C	H	N		
	234 (7,700) 299 (4,600)		3250 2700 2580 2340	1646 1625	1518								100	10
	f232 (7,850) 300 (4,750)	217 (31,650) 240 (11,550) f268 (2,250) 329 (6,650)	3250 3210 3160sh	1640	1545	$C_{10}H_{14}N_2O_2$	61·83	7·27	14·42	61·99	7·35	14·45	650	79[e]
	213 (36,900) 262 (11,200)		3320 3290 3200	1655sh 1640	1530								300	6
	214 (37,350) 262 (10,650)		3280sh 3200 3060	1625	1545 1535	$C_{13}H_{20}N_2O_4$	58·19	7·51	10·44	58·13	7·60	10·64	650	27[e]
	214 (36,000) 262 (10,600)		3400	1635	1530	$C_{13}H_{20}N_2O_6$—½H_2O	50·48	6·84	9·07	50·52	7·16	9·13		56

	265 (4,750) f304 (1,000)	246 (4,150) 323 (5,800)	3360 3210 3080	1658	1577 1555	$C_9H_{12}N_2O_3$	51·17	6·20	19·90	51·10	6·17	19·95	>1000	8×10^{-5}
	234 (48,700) f272 (8,650) 283 (7,700) 294 (6,050) 353 (2,150)		3280 3180 3100sh	1660	1565 1590 1510								sl >1000	0[e]
	235 (48,300) f272 (7,900) 295 (6,200) f334 (1,900) 356 (2,000)	225 (10,800) 252 (29,600) f280 (4,300) 292 (3,300) 304 (1,500)	3350 3260 3080	1695 1635sh	1565 1550	$C_{19}H_{16}N_2O_2$	68·83	6·60	11·47	68·19	6·57	11·32	>1000	68[e]
	See Experimental Section												553	0
	220 (37,100) f224 (5,750) 281 (6,150) 289·5 (5,250)		3200 3060	1167 1643	1562 1535	$C_{13}N_{17}N_3O$	67·50	7·91	18·17	67·78	7·73	18·36	650	18[e]

[a] Prepared according to Bondi[13]; m.p. 145°.
[b] For synthesis see Experimental section.
[c] Prepared according to Pearl and Beyer[14]; hemihydrate, m.p. 157–158°; Pepe[15]; anhydrous compound, m.p. 159°. Our sample was crystallized from methanol and analyzed for the anhydrous material.
[d] Prepared according to Seligman *et al.*[16]; m.p. 212–213°.
[e] Run in suspension.

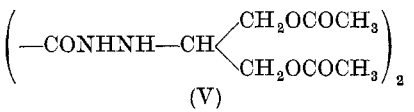
C. Special cases. Formation of the tetraacetate (V) by direct acetylation of compound 25 (Table I) proved difficult, very likely because of acetyl O→N migration to give the *N*,*N*-diacetyltetraacetate (compound 26).



(25) (26)

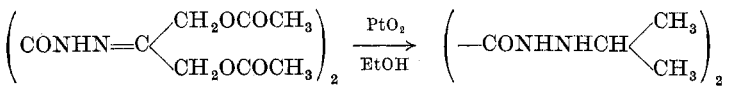
Compound 26 behaved as a monacidic substance on titration with aqueous sodium hydroxide. This was not due to hydrolysis of one of the *N*-acetyl groups since some of the unchanged compound 26 could be isolated after treatment with one equivalent of base, but rather to the acidity of the system $(—CONHNRCOCH_3)_2$.

When compound 25 was treated with perchloric acid and then subjected to acetylation, the perchlorate of the desired tetraacetate (V) was isolated.



(V)

Compound V could not be obtained by attempted hydrogenation described below, since hydrogenolysis took place very readily:



(8) (Table I)

In the case of compounds 28 and 29 (Table I), the desired hydrazine (VI) was prepared from hydrazine hydrate and

$$NH_2NH_2 + CH_2{=}CHCONHCH_2C_6H_5 \longrightarrow NH_2NHCH_2CH_2CONHCH_2C_6H_5 \quad (VI)$$

N-benzylacrylamide and used without purification. Since an excess of hydrazine was used in order to obtain a better yield of the mono- rather than the bis-adduct, a corresponding excess of methyl hydrazidooxalate or diethyl oxalate (as shown in the equations below) was employed during the condensation in order to react with the residual hydrazine. Fortunately the solubilities of the desired bishydrazides (28) and (29) were quite different from that of the by-product, oxalic acid bishydrazide, so that separation by crystallization could be easily achieved.

$$\begin{matrix} CONHNH_2 \\ | \\ 3COOCH_3 \end{matrix} + 3NH_2NH_2 + CH_2{=}CHCONHCH_2C_6H_5 \longrightarrow \begin{matrix} CONHNH_2 \\ | \\ CONHNH\cdot CH_2CH_2CONHCH_2C_6H_5 \end{matrix} + 2(CONHNH_2)_2 \quad (28)$$

$$\begin{matrix} COOEt \\ | \\ 3COOEt \end{matrix} + 6NH_2NH_2 + 2CH_2{=}CHCONHCH_2C_6H_5 \longrightarrow (CONHNHCH_2CH_2CONHCH_2C_6H_5)_2 + 2(CONHNH_2)_2 \quad (29)$$

Pharmacology

Monoamine oxidase activity in vitro. Monoamine oxidase activity was determined by the method of Bhagvat *et al.*,[19] using the Warburg apparatus. Vessels of about 15 ml capacity with one side bulb were used. The source of enzyme was guinea-pig liver which was homogenized in Na-K phosphate buffer pH 7·4, 0·25M. Each vessel contained 1 ml of homogenate, 0·1 ml of NaCN M/15, 0·5 ml of buffer and 0·2 ml of water for the control or 0·2 ml of the solution of the compound to be tested in a concentration of 10^{-2}M.

Serotonin (0·2 ml, $6{\cdot}2 \times 10^{-2}$M) was placed in the side bulb.

The centre well contained 0·1 ml of 0·002N KOH solution and 0·1 ml of 0·02M NaCN solution and a small piece of fluted Whatman No. 1 filter paper. The gas phase was air. After 10 min equilibration in the bath at 37°, the substrate was tipped into the main compartment and the manometer was read at 10 min intervals for 1 h.

The values for monoamine oxidase inhibition at 10^{-3}M are the results of a screening procedure in which compounds were tested at this concentration in triplicate. When inhibitions significantly greater than 50 per cent at 10^{-3}M were found, $[I]_{50}$ determinations were done by testing at a number of different concentrations so that concentrations inhibiting both more and less than 50 per cent were found. The results were plotted on semi log paper and the concentration inhibiting 50 per cent was determined.

A number of compounds tested were not completely soluble in the medium used. These were ground thoroughly and added as a fine suspension to the Warburg vessel. It is thus possible that a number of compounds showing little or no inhibition were ineffective by virtue of their insolubility. The $[I]_{50}$ values are probably reliable for the conditions of our experiment since more dilute solutions were used and since a dose-response curve could be obtained.

Monoamine oxidase activity in vivo. Several compounds were tested in rats (one rat for each compound) in preliminary acute experiments for oral activity. The compounds were dissolved or suspended in water (2·5 mg/ml) and given by gastric intubation in a dose of 25 mg/kg to rats which had been fasted 18 h. The rats were decapitated 3 h later and the liver and brain were removed, weighed and homogenized as described above for MAO activity determinations *in vitro*. The quantity of brain used was 400 mg/ml and that of liver 200 mg/ml. One ml of these homogenates was used. The results of these *in vivo* experiments are shown in Table IV. The average of 20 untreated normal rats served as control values.

From Table IV it may be seen that of the compounds tested No. 25 and No. 24 showed little or no inhibitory effect in the doses given. This may mean either that the compounds were not absorbed through the gastrointestinal tract or that they were rapidly metabolized. Of the remaining three compounds, all

Table IV. Monoamine oxidase activity after oral dosage of rats with several derivatives of oxalic acid hydrazides

Compound no.	Inhibition, %	
	Liver	Brain
6[a]	100	100
8	100	100
15	100	14
16	100	14
17	100	100
24	26	0
25	0	0

[a] See Table I for the structures of these compounds.

showed marked MAO inhibitory activity in the liver while three showed marked inhibition of the brain.

Four compounds were tested in a chronic experiment in which rats (seven rats for each compound) were dosed daily p.o. for about 2 weeks. On alternate days a rat in each group was decapitated and MAO activity determined on the brain and liver. The results are shown in Table V.

It may be seen that compounds 6, 8 and 17 caused good inhibition of MAO in brain whereas compound 15 had little effect in this organ. Since the liver, but not the brain, MAO was inhibited by this last compound it would appear that it does not readily cross the blood–brain barrier.

Structure–Activity Relationship

In the series of oxalic acid bishydrazides (Table I), the parent compound (1) was inactive as an MAO inhibitor. When N^2 and $N^{2'}$ were substituted by a phenyl group (22) or two methyl groups (20) or phenyl and methyl groups (23), no activity resulted.

N^2,$N^{2'}$-dialkyl and diaralkyl oxalic acid bishydrazides were active, except the first member of the series (5). The most active were the N^2,$N^{2'}$-diethyl, diisopropyl, and diphenylethyl (6, 8, 15) compounds. Substitution by hydroxyl groups tended to decrease

Table V. Percentage inhibition of MAO activity *in vivo* in chronic experiments

Compound no.	Oral daily dose, mg/kg	Tissue	Number of doses													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
8	1·5	Liver	77		89		100		100		99		92		95	
		Brain	74		76		100		100		100		100		100	
	0·5	Liver		73		78		93		83		86		89		80
		Brain		48		61		100		100		100		100		100
17	0·5	Liver	48		74		83		76		81		89		84	
		Brain	32		50		54		70		91		91		91	
	0·25	Liver	13		60		61		60		27		81		56	
		Brain	9		20		13		29		5		52		77	
15	0·5	Liver	15		58		58		63		66		75		66	
		Brain	0		5		13		0		20		29		13	
	0·25	Liver	14		41		24		20		30		56		30	
		Brain	0		0		0		0		0		0		0	
6	0·5	Liver	30		51		64		71		87		85		89	
		Brain	0		64		89		89		100		100		100	
	0·25	Liver	0		38		19		24		40		33		47	
		Brain	9		7		7		16		25		41		79	

the toxicity (compounds 27 and 12; 25 and 8) but, at least in the case of compound 25, oral activity was lost.

Stereochemical considerations seemed to be important in the case of the two diastereoisomers (18 and 19). Compound 19 had fair activity while 18 had none.

When the bishydrazide was monosubstituted, activity decreased in the case of compounds 2 and 4 but was retained in compounds 3 and 28. In the case of disubstitution by different groups, the activity decreased in the case of compound 9, but was retained in 17 and 24. We have not studied compounds that carried groups on N^1 and $N^{1'}$, except compound 26.

In the series of the various aliphatic hydrazides (Table II), inactive compounds resulted when one C=O function was eliminated from the above general structure [see 2,2′-bis(isopropyl)-carbohydrazide] or when the two C=O functions were separated by a methylene group [see malonic acid bis(2-isopropyl)hydrazide]. However, separation by two methylene groups again restored activity [see succinic acid bis(2-isopropyl)hydrazide].

Of the aromatic hydrazides in Table III, N^2-(1-hydroxymethyl-2-hydroxyethyl) isonicotinic acid hydrazide showed considerable activity.

In investigating the mode of action of 1-isonicotinoyl-2-isopropylhydrazine on MAO, Barsky *et al.*[20] observed that while hydrazine was without effect, the incorporation of an alkyl residue (e.g. ethyl), but not of an acyl group, led to the formation of a very active MAO inhibitor. There was one exception to the alkylation rule in that methylation did not produce a strong inhibitor. He concluded that the part of iproniazid which was essential for inhibition was the moiety indicated below:

Davison[21] found that the inhibition of iproniazid required oxygen and he postulated that irreversible inhibition was the result of a dehydrogenation of iproniazid at the active centre of the enzyme. For this reaction a free H on the N^2 was essential.

Our results agree with these but show that the nature of the acyl group is important in the group of hydrazides which we studied.

Experimental*†

Condensation of ethyl indole-3-glyoxylate with hydrazine. A mixture of ethyl indole-3-glyoxylate (50·0 g, 0·23 mole) and hydrazine hydrate (120 ml) was refluxed for 5·5 h. The resulting solution was allowed to stand overnight and then evaporated to dryness. The oily residue was crystallized from methanol (25 ml) and water (170 ml) to give 32 g (73·5 per cent) of indole-3-acethydrazide, m.p. 137·5–141°. Two crystallizations from benzene–methanol afforded clusters of needles, m.p. 143·5–144·5°.‡ A mixture melting point with an authentic sample showed no depression. Ultraviolet spectrum showed $\lambda_{max.}$ 219 (35,700); 273 (5,850); 280 (6,200); 289 (5,350). No change in base. Infrared spectrum showed NH: 3310, 3220, 3100 sh; C=O: 1667; 1630 sh; C=C: 1608, 1573, 1492; amide II: 1548, 1532.

Anal. Calcd. for $C_{10}H_{11}N_3O$: C, 63·47; H, 5·86; N, 22·21. Found: C, 63·33; H, 5·64; N, 21·68.

A solution of the hydrazide (1·89 g, 0·01 mole) in water (25 ml) containing sodium hydroxide (4 g) was refluxed for 4 h. It was then cooled, filtered and acidified to give 1·6 g (91·5 per cent) of indole-3-acetic acid, m.p. 168–169° (d.). A mixture melting point with an authentic sample [m.p. 168–169·5° (d.)] showed no depression.

Condensation of ethyl 2-methylindole-3-glyoxylate with hydrazine. A suspension of ethyl 2-methylindole-3-glyoxylate (50 g, 0·216 mole) in hydrazine hydrate (120 ml) was refluxed for 5·5 h. The suspension was cooled and filtered (original filtrate), and the yellow solid was washed with water to give 24·8 g (49·5 per cent) of the hydrazone hydrazide; m.p. 192–195°, resolidified, m.p. 306–313°. It was crystallized from pyridine (75 ml) and ether (100 ml) and melted at 209–210°, resolidified, m.p. 305–311° (unchanged on

* Melting points are taken in a capillary tube and are uncorrected. Ultraviolet spectra (recorded in mμ) were determined in 95 per cent ethanol using a Cary spectrophotometer, Model 14. Infrared spectra (recorded in cm^{-1}) were determined in Nujol using a Perkin–Elmer recording infrared spectrophotometer Model 21.

† The authors are indebted to Mr. W. A. Struck and his associates for microanalyses, to Dr. R. W. Rinehart and Mr. M. F. Grostic for infrared and ultraviolet spectra, to Mr. W. Veldkamp for the LD_{50} values, to Mr. R. A. Walk for monoamine oxidase assays and to Mr. L. G. Laurian for laboratory assistance.

‡ Reported m.p. 138–139°.[22]

further recrystallization). Ultraviolet spectrum (in 1 per cent dimethylformamide–ethanol) showed f 244 (9,250); $\lambda_{max.}$ 269 (13,100); f 286 (9,950). Infrared spectrum showed NH: 3360, 3280 sh, 3220 sh, 3170; C=O: 1637 sh, 1630; C=C: 1606, 1573, 1545, 1503.

Anal. Calcd. for $C_{11}H_{13}N_5O$: C, 57·13; H, 5·67; N, 30·29. Found: C, 57·51; H, 5·53; N, 29·10.

The original filtrate was evaporated to dryness and the residue was triturated with methanol (25 ml) to give 3·6 g (14 per cent) of oxalic acid bishydrazide, efferv. 162°, resolidified, m.p. 230–233°. After two recrystallizations from water it melted at 243–244°.

Anal. Calcd. for $C_2H_6N_4O_2$: C, 20·34; H, 5·12. Found: C, 20·50; H, 5·00.

A mixture melting point with an authentic sample* (m.p. 245–246°) showed no depression. The ultraviolet and infrared spectra were identical.

Condensation of 3-indoleglyoxylyl chloride with hydrazine. Oxalyl chloride (81 g, 0·645 mole) was added to a solution of indole (58·5 g, 0·5 mole) in ether (700 ml) during 15 min with occasional cooling so that only mild refluxing occurred. The suspension was then stirred at room temperature for 1 h. The resulting yellow solid was filtered and washed with ether, followed by Skelly B.† The solid was added portionwise during 45 min to a solution of anhydrous hydrazine (37 ml) in ether (500 ml). The temperature was maintained at 25° by occasional cooling in ice. The mixture was then stirred at room temperature for 4 h. The solid was filtered, washed with ether and then with water. It was refluxed with ethanol (4 l.), and the undissolved material was filtered and washed with hot ethanol to give 35·8 g of 1,2-bis(3-indoleglyoxylyl)-hydrazine, m.p. > 300°. A sample was crystallized for analysis from pyridine, m.p. > 335°. Ultraviolet spectrum (in 1 per cent dimethylformamide–ethanol) showed f 248 (16,200); 256·5 (18,400); 268 (18,100); 274·5 (16,700); 330 (17,800). Infrared spectrum showed NH: 3340 sh, 3240 sh, 3190, 3210 sh; bonded NH: 2680; C=O/C=C: 1630 sh, 1615 sh, 1597, 1570 sh, 1522, 1500; ring: 800 sh, 790, 780, 760, 752 sh, 747, 660.

* Prepared according to Borsche *et al.*[1]

† Trade name for petroleum ether (b.p. 60–68°).

Anal. Calcd. for $C_{20}H_{14}N_4O_4$: C, 64·17; H, 3·77; 14·97. Found: C, 64·11; H, 3·76; N, 15·26.

Syntheses of Compounds Described in Table I

Oxalic acid hydrazide 2-isopropylhydrazide (*2*). A solution of hydrazine hydrate (15 g, 0·3 mole) in ethanol (25 ml) was added during 15 min to a solution of diethyl oxalate (75 g, 0·515 mole) in ethanol (25 ml), keeping the inside temperature at $-15°$ to $-20°$.[23-25] The resulting suspension was stirred in the cold for 10 min, then at room temperature for 1·25 h. It was then filtered to separate oxalic acid bishydrazide and the filtrate was evaporated *in vacuo* at 30–35°. Water (75 ml) was added during cooling and the solution was extracted twice with ether; the aqueous solution was evaporated at 35° to remove all ether. Acetone (44 ml) was added and the solution was allowed to stand for 1 h. It was evaporated *in vacuo* at 25–30° overnight; the resulting solution was freeze-dried to give an oily colourless solid, acetone (250 ml) was added and the solution was refluxed for 2 h. It was evaporated at 30° to give 37·7 g of the crude isopropylidene derivative, m.p. 45–54° (hygroscopic), which could not be easily purified and which gave poor analytical results. Ultraviolet spectrum showed $\lambda_{max.}$ 253 (5,750; f 328 (530)). Infrared spectrum showed ester C=O: 1745; C=O: 1695, 1685–1650; C=N: 1633, 1625.

A solution of the crude isopropylidene derivative (37·0 g, 0·215 mole) in ethanol (200 ml) and platinum oxide (1 g) was hydrogenated at an initial pressure of 52 lb.* A further 1 g of catalyst was added after about two-thirds of the required amount was absorbed. The resulting yellow solution was evaporated to dryness at 40° to give 36 g of the isopropyl compound as an oil. A solution of this crude derivative (36·0 g, 0·206 mole) in ethanol (50 ml) was added during 10 min to a solution of hydrazine hydrate (12·7 g, 0·227 mole) in ethanol (100 ml). The resulting suspension was stirred for 1 h and filtered, and the solid was washed with ethanol (28 g, m.p. 172–195°). It was refluxed with ethanol

* All the hydrogenation experiments described in this article were run at initial pressure of about 50 lb of hydrogen and at room temperature unless otherwise specified. Platinum oxide was used as the catalyst.

(2,250 ml), filtered from an insoluble precipitate and allowed to crystallize from this volume; 17·5 g of ill-defined clusters, m.p. 178–181·5°, was obtained. The second crop amounted to 2·9 g, m.p. 172–174°. The analytical sample (from ethanol) melted at 180·5–182°.

Oxalic acid hydrazide phenethylhydrazide (*3*). Ethyl hydrazidooxalate was prepared from diethyl oxalate (126 g, 0·86 mole) and 25 g (0·5 mole) of hydrazine hydrate as described above.

The aqueous solution of ethyl hydrazidooxalate was added dropwise to a solution of phenethylhydrazine (68 g, 0·5 mole, prepared as described below under compound 15) in methanol (150 ml). The resulting cloudy solution was refluxed for 30 min during which time a thick suspension resulted. It was cooled and filtered and the solid washed with methanol. The product was crystallized from water (1500 ml) to give 42·8 g of material, m.p. 151° (cloudy melt), 162° (clear oil). Recrystallization from the same volume of water afforded 37·7 g (33 per cent yield); m.p. 153°, resolidified, clear melt at 173°. The analytical sample was obtained by recrystallization from water and showed the same melting point.

Oxalic acid hydrazide 2-(α-benzyl)-ethylhydrazide (*4*). *A. Methyl hydrazidooxalate.* A solution of hydrazine hydrate (50 g, 1 mole) in methanol (125 ml) was added during 1 h to a solution of dimethyl oxalate (130 g, 1·1 mole) in 1250 ml of methanol (inside temperature, −15°). The resulting suspension was stirred in the cold for 15 min, then at room temperature for 2 h. The suspension was filtered to separate oxalic acid bishydrazide and the filtrate was concentrated at 30° *in vacuo* until crystallization commenced; 46·9 g (40 per cent yield), m.p. 113–114°. The analytical sample melted at 115–116° (from methanol).[23] Ultraviolet spectrum: 249·5 (5,150); in base: 250 (5,800). Infrared spectrum: NH: 3300, 3240, 3200, 3130, 3020; C=O: 1740, 1700, 1620; amide II: 1555.

Anal. Calcd. for $C_3H_6N_2O_3$: C, 30·51; H, 5·12; N, 23·72. Found: C, 30·79; H, 5·09; N, 24·32.

B. Methyl 1-benzylethylidene hydrazidooxalate. Phenylacetone (54 g, 0·4 mole) was added to a solution of methyl hydrazidooxalate (23·6 g, 0·2 mole) in methanol (200 ml) and the solution was refluxed for 1·75 h. It was then evaporated, initially *in*

vacuo and finally at 0·1 mm, on a steam bath to give a yellow oil which was used directly for the next step.

C. Methyl 2-(α-benzyl)-ethylhydrazidooxalate. A solution of the crude 1-benzylethylidene compound (0·2 mole) in methanol (200 ml) was hydrogenated as described in the case of compound 2 to give 47·8 g of a yellow oil.

D. Compound 4. A solution of the α-benzylethyl compound (0·2 mole) in methanol (100 ml) was added during 10 min to a solution of hydrazine hydrate (15·0 g, 0·3 mole) in methanol (200 ml). The suspension was stirred for 2 h, and the product was filtered and refluxed with 2 l. of ethanol. The cloudy mixture was filtered from some undissolved material, evaporated down to about 1 l. and allowed to crystallize to give 31·0 g of crude product. This solid (31 g) was extracted with ethanol (750 ml) and the solution filtered from insoluble material and allowed to crystallize; 23 g, m.p. 152–153° (sintering at 140°). Recrystallization from 900 ml of water gave 19 g, m.p. 153–157°. Another recrystallization from 1 l. of water gave 15·5 g, m.p. 150–152° (opalescent gel), clear oil at 158°. The analytical sample was obtained by one further recrystallization from water; well-defined plates, m.p. 158–159° (sintering at 142°).

*Oxalic acid bis(2-ethyl)-hydrazide (6). A. Oxalic acid bis-ethylidene hydrazone.** Acetaldehyde (35·2 g, 0·8 mole) was added to an ice-cooled suspension of 23·6 g (0·2 mole) of oxalic acid bishydrazide in 800 ml of absolute ethanol. The mixture was stirred at room temperature for 1 h, then heated (inside temperature 65–70°) for 6 h. The suspension was filtered to give 32·5 g (96 per cent) of product, m.p. 259° (efferv.), unchanged on recrystallization from dimethylformamide. Ultraviolet spectrum showed $\lambda_{max.}$ 252 (18,550); infrared spectrum showed NH: 3180, 3020; C=O: 1663; C=N: 1624; amide II: 1530.

Anal. Calcd. for $C_6H_{10}N_4O_2$: C, 42·35; H, 5·92; N, 32·93. Found: C, 42·37; H, 6·03; N, 32·56.

B. Compound 6. A suspension of the hydrazone (3·4 g, 0·02 mole) in ethanol (200 ml) was hydrogenated for 4·5 h at 50° in the presence of platinum oxide (0·2 g). The resulting mixture was filtered hot and the filtrate was evaporated to about half its volume and allowed to crystallize; 1·13 g (32·6 per cent), m.p.

* This compound was reported previously without details.[25]

202·5–204°. Recrystallization from ethanol raised the melting point to 203·5–205·5°.

This compound had been prepared previously by condensation of diethyl oxalate with ethylhydrazine.[27]

Oxalic acid bis-(2-propyl)-hydrazide (*7*). *A. Oxalic acid bis-propylidenehydrazone.** A mixture of oxalic acid bishydrazide (23·6 g, 0·2 mole), propionaldehyde (39 g, 0·8 mole) and ethanol (800 ml) was refluxed with stirring for 1·5 h. The resulting suspension was filtered to give 38·2 g (96·5 per cent) of product, m.p. 259·5° (d.). Crystallization from ethanol afforded needles, m.p. 260·5° (d.). Ultraviolet spectrum showed 253 (19,650); infrared spectrum showed NH: 3190; C=O: 1657; C=N: 1630; amide II: 1550, 1535.

Anal. Calcd. for $C_8H_{14}N_4O_2$: C, 48·47; H, 7·12; N, 28·28. Found: C, 48·77; H, 7·56; N, 27·90.

B. Compound 7. The hydrazone (3·96 g, 0·02 mole) was hydrogenated as described in the case of compound 6. Yield, 3·31 g (83 per cent), m.p. 193·5–194·5°, unchanged on recrystallization from ethanol.

Oxalic acid bis(2-isopropyl)hydrazide (*8*). *A. Oxalic acid bis-(isopropylidene)hydrazide.** The condensation was run as described above for compound 7 using 46·2 g (0·8 mole) of acetone. A solution resulted after 30 min. About 350 ml of solvent was distilled off and the solution was allowed to crystallize at room temperature for 2 h; yield, 36 g (91 per cent), m.p. 219–221°. Recrystallization from ethanol afforded colourless needles, m.p. 222·5–223·5°. Ultraviolet spectrum showed 258 (16,650); infrared spectrum showed NH: 2370 ; C=O: 1674 sh, 1665; C=N: 1637; amide II: 1508.

Anal. Calcd. for $C_8H_{14}N_4O_2$: C, 48·47; H, 7·12; N, 28·27. Found: C, 48·27; H, 7·16; N, 28·02.

B. Compound 8. (*a*) *Reduction in ethanol.* Oxalic acid bis-(isopropylidene)hydrazide (5·57 g, 0·0282 mole) was suspended in absolute ethanol (110 ml) and hydrogenated at 50° in the presence of 0·11 g of platinum oxide. The theoretical amount of hydrogen was absorbed in 2·5–3 h. The resulting suspension was filtered. The solid was dissolved in boiling ethanol (500 ml)

* This compound was previously reported by de Graaf[28] (m.p. 210°), and Nilsson[26] (no m.p. given).

and the solution was filtered, evaporated to about 400 ml and allowed to crystallize; yield, 4·16 g (73 per cent); m.p. (introduced at 150°) 194–195°, unchanged on further recrystallization.

(*b*) *Reduction in acetic acid.* The hydrogenation was carried out using a suspension of 1·98 g (0·01 mole) of the bisisopropylidene derivative in acetic acid (30 ml) and 0·11 g of platinum oxide; it was complete in one hour. The resulting solution was filtered. Ethanol (100 ml) was added to the filtrate and the crystalline precipitate was filtered to give 1·39 g (69·6 per cent) of a product melting at 191·5–192·5°. It was identical with the bisisopropyl compound described above (ultraviolet and infrared spectra, neutral equivalent).

Oxalic acid 2-ethylhydrazide-2-isopropylhydrazide (*9*). *A. Oxalic acid 2-isopropylhydrazide-ethylidenehydrazide.* Acetaldehyde (2·64 g, 0·06 mole, cooled in dry ice) was added all at once to an ice-cooled suspension of oxalic acid 2-isopropylbishydrazide (4·8 g, 0·03 mole) in ethanol (120 ml). The mixture was stirred for 2·25 h and the resulting thick suspension was refluxed for 20 min. It was cooled in ice and the solid was filtered and washed with ethanol; 2·95 g melting at 185–204° (fast) was obtained. The second crop amounted to 1·4 g, m.p. 189–193°. The analytical sample melted at 196–198° (from ethanol). Ultraviolet spectrum showed 249 (12,050); in 0·01N KOH: 241 (9,550); 278 (6,300). Infrared spectrum showed NH: 3220, 3160, 3000; 1720w, 1696w; C=O/C=N: 1660–1625 s; amide II: 1543.

Anal. Calcd. for $C_7H_{14}N_4O_2$: C, 45·15; H, 7·58; N, 30·09. Found: C, 45·17; H, 7·96; N, 30·16.

B. Compound 9. The ethylidene derivative (4·05 g, 0·0217 mole) in ethanol (200 ml) was hydrogenated as described in the case of compound 6 to give 2·5 g, m.p. 173–174°, unchanged on further crystallization. A second crop amounted to 0·35 g, m.p. 167–168°.

*Oxalic acid bis(2-*n*-butyl)-hydrazide* (*10*). *A. Oxalic acid bis-butylidene hydrazide.** A mixture of oxalic acid bishydrazide (5·9 g, 0·05 mole), butyraldehyde (14·4 g, 0·2 mole) and absolute ethanol (200 ml) was refluxed under nitrogen for 6 h. The suspension was cooled and filtered and the solid was washed with ethanol; it weighed 11 g (97·5 per cent), m.p. 263° (efferv.). Recrystallization from ethanol afforded colourless needles, m.p.

* This compound was reported previously[26] without details.

264° (efferv.). Ultraviolet spectrum showed $\lambda_{max.}$ 253 (18,850). Infrared spectrum showed NH: 3180, 3030; C=O: 1668; C=N: 1626; amide II: 1529.

Anal. Calcd. for $C_{10}H_{18}N_4O_2$: C, 53·08; H, 8·02; N, 24·76. Found: C, 53·21; H, 8·09; N, 24·85.

B. Compound 10. The hydrazone (2·26 g, 0·01 mole) was hydrogenated as described in the case of compound 6. Yield, 1·5 g (65 per cent), m.p. 157–158°, unchanged on further recrystallization from ethanol.

Oxalic acid bis(2-isobutyl)hydrazide (11). A. Oxalic acid bis-isobutylidenehydrazide. A suspension of oxalic acid bishydrazide (11·8 g, 0·1 mole) in ethanol (400 ml) and isobutyraldehyde (29·0 g, 0·4 mole) was refluxed with stirring for 3·5 h. The resulting suspension was filtered to give 20·6 g (88·5 per cent) of the product, m.p. 251–252°, unchanged on recrystallization from ethanol. Ultraviolet spectrum showed $\lambda_{max.}$ 254 (20,400). Infrared spectrum showed NH: 3180, 3010; C=O: 1660; C=N: 1628; amide II: 1530.

Anal. Calcd. for $C_{10}H_{18}N_4O_2$: C, 53·08; H, 8·02; N, 24·76. Found: C, 53·29; H, 8·21; N, 24·84.

B. Compound 11. The hydrazone (0·01 mole) was hydrogenated as described in the case of compound 6. Yield, 1·73 g (75 per cent), m.p. 164·5–165·5°. Recrystallization from ethanol afforded material melting at 165–166°.

*Oxalic acid bis[2-(1-methyl)-propyl]hydrazide (12). A. Oxalic acid bis(2-s-butylidene)hydrazide.** A mixture of oxalic acid bishydrazide (23·6 g, 0·2 mole), methyl ethyl ketone (57·5 g, 0·8 mole) and ethanol (800 ml) was refluxed for 2·5 h. A solution resulted after 1 h. The solvent (600 ml) was evaporated and the solution was allowed to crystallize. The product was filtered and washed with ether; yield, 31·4 g (70 per cent) of colourless needles, m.p. 168·5–170°. An analytical sample melted at 169–170° (from ethanol). Ultraviolet spectrum showed $\lambda_{max.}$ 258 (16,750). Infrared spectrum showed NH: 3230; C=O: 1660; C=N: 1630; amide II: 1500.

Anal. Calcd. for $C_{10}H_{18}N_4O_2$: C, 53·08; H, 8·02; N, 24·76. Found: C, 53·42; H, 8·14; N, 24·26.

B. Compound 12. The bishydrazone (31·1 g, 0·137 mole) in

* This compound was reported previously[26] without details.

ethanol (300 ml) was hydrogenated as described in the case of compound 6. Yield, 28 g (89 per cent) of colourless plates, m.p. 135–136·5°, unchanged on further recrystallization from ethanol.

Oxalic acid bis[2-(1-ethylpropyl)]hydrazide (13). A. Oxalic acid bis(1-ethylpropylidene)hydrazide. A suspension of oxalic acid bishydrazide (11·8 g, 0·1 mole) in absolute ethanol (400 ml) and 34·4 g (0·4 mole) of diethyl ketone was refluxed with stirring for 7 h; solution resulted after 3 h. It was allowed to crystallize overnight and filtered to give 17·7 g of product melting at 182–183°. A second crop amounted to 4·5 g, m.p. 181–182°; total yield, 87·5 per cent. A sample was recrystallized for analysis from ethanol; needles, m.p. 182–183°. Ultraviolet spectrum showed $\lambda_{max.}$ 259 (16,950). Infrared spectrum showed NH: 3230; C=O: 1665; C=N: 1633; amide II: 1505.

Anal. Calcd. for $C_{12}H_{22}N_4O_2$: C, 56·67; H, 8·72; N, 22·03. Found: C, 56·58; H, 8·66; N, 21·78.

B. Compound 13. The bishydrazone (2·54 g, 0·01 mole) was hydrogenated as described in the case of compound 6. Yield, 2·29 g (89 per cent), m.p. 117–119°. Recrystallization from ethanol afforded leaflets melting at 118·5–119·5°.

Oxalic acid bis(2-benzyl)hydrazide (14). Benzylhydrazine was prepared according to Biel *et al.*[29] Diethyl oxalate (9·75 g, 0·0666 mole) was added dropwise to a stirred solution of benzylhydrazine (24·4 g, 0·2 mole) in methanol (150 ml). The solution was refluxed for 1 h and the resulting suspension was cooled and filtered. The product was recrystallized from ethanol to give colourless needles; yield, 16·5 g (84 per cent), m.p. 165–167° unchanged on further recrystallization.

Oxalic acid bis(phenethylhydrazide) (15). A. Preparation of phenethylhydrazine.[30] Phenethyl bromide (185·1 g, 1 mole) was added during 0·5 h to a stirred refluxing solution of hydrazine hydrate (600 g, 12 moles) in ethanol (800 ml). The solution was refluxed for 22 h. It was then evaporated to a small volume *in vacuo*.* The residual oil was extracted with ether (5 × 250 ml) and the cloudy ether solution was shaken with solid potassium carbonate until clear. It was then filtered and evaporated, and

* If all the solvent is taken off at this stage, addition of ether causes precipitation of a solid and necessitates the addition of water during work-up, which lowers the yield considerably.

the resulting oil was distilled; b.p. 93–95°/0·5 mm, yield, 98·8 g (72 per cent).

B. Compound 15. Diethyl oxalate (7·9 g, 0·054 mole) was added during 5 min to a solution of phenethylhydrazine (22·09 g, 0·162 mole) in methanol (50 ml). The solution was refluxed for 30 min, and allowed to crystallize overnight. The product was filtered and washed with methanol, and crystallized from ethanol to give 15·1 g (86 per cent), melting at 163–164°. An analytical sample melted at 164–165°.*

Oxalic acid bis(α-methyl-phenethylhydrazide) (16). A. Oxalic acid bis(α-methylphenethylidene)hydrazone. A mixture of oxalic acid bishydrazide (5·9 g, 0·05 mole), phenylacetone (26·8 g, 0·2 mole) and absolute ethanol (200 ml) was refluxed with stirring for 7 h. The suspension was filtered to give 16·8 g (96 per cent) of needles, m.p. 208–209°, unchanged on crystallization from ethanol. Ultraviolet spectrum showed $\lambda_{max.}$ 259 (21,150); f 264 (20,750); f 268·5 (19,600). Infrared spectrum showed NH: 3280; C=O: 1688, 1680 sh; C=N: 1635; C=C: 1600, 1583, 1505, 1498.

Anal. Calcd. for $C_{20}H_{22}N_4O_2$: C, 68·55; H, 6·33; N, 15·99. Found: C, 68·70; H, 6·44; N, 16·17.

B. Compound 16. The hydrazone (3·5 g, 0·01 mole) was hydrogenated as described in the case of compound 6. Yield, 2·9 g (82 per cent); needles, m.p. 159–160°, unchanged on further recrystallization.

Oxalic acid 2-isopropylhydrazide-2-(α-methylphenethyl)hydrazide (17). A. Oxalic acid 2-isopropyl-α-benzylethylidene bishydrazide. Phenylacetone (8·1 g, 0·06 mole) was added to a solution of oxalic acid 2-isopropylbishydrazide (4·8 g, 0·03 mole) in ethanol (400 ml) and refluxed for 2 h. The solution was evaporated to *ca.* 100 ml and allowed to crystallize. The product was filtered and washed with ether; yield 4·2 g, m.p. 142·5–143·5°, unchanged on recrystallization. A second crop amounted to 2·3 g of the same m.p.; total yield, 78 per cent. Ultraviolet spectrum showed $\lambda_{max.}$ 255 (13,900); f 204 (12,900); f 268 (11,600). Infrared spectrum showed NH: 3265, 3220; =CH: 3020; C=O/C=N: 1690, 1655, 1642, 1633; C=C: 1603 sh, 1585, 1495 sh, 1490; amide II: 1548, 1515, 1507.

* Reported[30] m.p. 161–162°.

Anal. Calcd. for $C_{14}H_{20}N_4O_2$: C, 60·85; H, 7·30; N, 20·28. Found: C, 61·91; H, 7·30; N, 19·99.

B. Compound 17. A solution of the α-benzylethylidene compound (6·2 g, 0·0224 mole) in ethanol (250 ml) was hydrogenated with 0·5 g of platinum oxide. The resulting suspension was heated, filtered, evaporated to about 150 ml and allowed to crystallize; yield 3·51 g, m.p. 150–153°, clear at 158°. An analytical sample melted at 156–159° (poorly defined clusters from ethanol).

Oxalic acid bis[2-(1-cyclopropyl)-ethyl]hydrazide (18 and 19). A. Oxalic acid bis(1-cyclopropyl)-ethylidene hydrazone. A mixture of oxalic acid bishydrazide (23·6 g, 0·2 mole), methyl cyclopropyl ketone (67·0 g, 0·8 mole) and ethanol (800 ml) was refluxed with stirring for 8 h. The resulting solution was allowed to stand overnight and the solid was filtered and washed with ethanol; yield, 39·3 g (79 per cent), m.p. 184–184·5°. An analytical sample melted at 184·5–185° (from ethanol). Ultraviolet spectrum showed $\lambda_{max.}$ 269 (20,450). Infrared spectrum showed NH: 3220; C=O: 1658; C=N: 1622; cyclopropyl: 1034, 1017, 997.

Anal. Calcd. for $C_{12}H_{18}N_4O_2$: C, 57·58; H, 7·25; N, 22·39. Found: C, 57·71; H, 7·51; N, 22·59.

B. Compounds 18 and 19. A suspension of the bishydrazone (18·3 g, 0·0732 mole) in ethanol (300 ml) and 1 g of platinum oxide was hydrogenated for 2·5 h. The resulting suspension (combined two runs of the above size) was diluted with ethanol (1500 ml), refluxed, filtered, and allowed to crystallize; yield 13·4 g, m.p. 188–190°. It was recrystallized from ethanol to give 9·8 g of isomer I, m.p. 191–192°. The analytical sample (from ethanol) melted at 193–194°.

The original filtrate was allowed to stand at room temperature for 2 days and deposited well-defined needles of isomer II, m.p. 163–164° (14·4 g) unchanged on further purification; total yield: 69 per cent.

*Oxalic acid bis(2-cyclopentyl)hydrazide (21). A. Oxalic acid biscyclopentylidene hydrazide.** A mixture of oxalic acid bishydrazide (11·8 g, 0·1 mole), cyclopentanone (33·6 g, 0·4 mole) and absolute ethanol (400 ml) was refluxed with stirring for 5 h. The resulting suspension was filtered to give 22·58 g (90 per cent) of material melting at 237–238°. Recrystallization from ethanol

* This compound was reported previously[26] without details.

afforded rods melting at 241–242° (d.). Ultraviolet spectrum showed $\lambda_{max.}$ 260 (18,550). Infrared spectrum showed NH: 3250; C=O, C=N: 1677 sh, 1665, 1660, 1645; amide II: 1500.

Anal. Calcd. for $C_{12}H_{18}N_4O_2$: C, 57·58; H, 7·25; N, 22·39. Found: C, 57·42; H, 7·43; N, 22·36.

B. Compound 21. The hydrazone (11·04 g, 0·044 mole) in absolute ethanol (300 ml) was hydrogenated as described in the case of compound 6. Yield, 7 g (62·5 per cent) of leaflets melting at 212–214°, unchanged on further recrystallization.

Oxalic acid 2-isopropylhydrazide-2-(α-hydroxymethyl-β-hydroxy)-ethylhydrazide (24). A solution of freshly distilled (see below) 1,3-dihydroxyacetone (5·4 g, 0·06 mole) in ethanol (20 ml) was added to a suspension of oxalic acid hydrazide 2-isopropylhydrazide (compound 2, 4·8 g, 0·03 mole) in ethanol (350 ml) and refluxed under nitrogen for 1·1 h. The initially formed pink solution turned yellow. The solution was evaporated to 100 ml and hydrogenated in the presence of 0·5 g of platinum oxide. It was filtered, evaporated to 50 ml and allowed to crystallize in the cold; 2·3 g, m.p. 130–135°. The analytical sample melted at 145–148° (from ethanol).

Oxalic acid bis{2-[2-hydroxy-1-(hydroxymethyl)-ethyl]hydrazide} (25). A. Oxalic acid bis[(2,2'-dihydroxy)-isopropylidene]hydrazide. 1,3-Dihydroxyacetone[31,32] (Aldrich) was freshly distilled from an oil-jacketed flask at 150°/0·1 mm. The distillate solidified and was melted before dissolving in ethanol for use in the reaction.

A mixture of oxalic acid bishydrazide (11·8 g, 0·1 mole), dihydroxyacetone (36·4 g, 0·4 mole) and ethanol (800 ml) was refluxed with stirring for 2·25 h under nitrogen. The resulting solution was allowed to stand overnight and the solid was filtered and washed with ethanol, then with ether. It was crystallized from dimethylformamide (250 ml) and ethanol (500 ml) to give 10·6 g of product, m.p. 170° (d.), unchanged on recrystallization. The second crop amounted to 5·7 g; total yield: 62 per cent. Ultraviolet spectrum showed $\lambda_{max.}$ 259 (19,050). Infrared spectrum showed NH/OH: 3330, 3200; C=O: 1679; amide II: 1507; C—O: 1089, 1028.

Anal. Calcd. for $C_8H_{14}N_4O_6$: C, 36·64; H, 5·38; N, 21·37. Found: C, 37·03; H, 5·65; N, 21·64.

B. Compound 25. A suspension of the bishydrazone (13·53 g,

0·0515 mole) in ethanol (300 ml) and platinum oxide (0·6 g) was hydrogenated at 50° for 2 h. The resulting suspension was filtered and the precipitate was crystallized from dimethylformamide (*ca.* 50 ml) and ethanol (200 ml) to give 11 g (80·4 per cent), m.p. 175·5–176·5°, unchanged on further purification.

Oxalic acid bis{2-acetyl-2-[2-acetoxy-1-(acetoxymethyl)-ethyl]-hydrazide} (26). A. With pyridine and 8 moles of acetic anhydride. The suspension of compound 25 (2·67 g, 0·01 mole) in pyridine (10 ml) and acetic anhydride (7·6 ml, 0·08 mole) was heated on a steam bath with stirring for about 2 min. The resulting brown solution was allowed to stand at room temperature overnight. It was then evaporated to dryness *in vacuo* on the steam bath to give a brown solid. Crystallization from water (20 ml) afforded 3·89 g (87·7 per cent) of compound 26 as colourless needles, m.p. 187–188°, unchanged on further recrystallization from water. The acetyl determination showed considerable degree of variability. A nuclear magnetic resonance spectrum* was in accord with the proposed structure.

B. With pyridine and 4 moles of acetic anhydride. This reaction was carried out as described under (*A*) but using acetic anhydride (4·2 ml, 0·44 mole) to give 0·66 g (15·2 per cent), m.p. 184–185°, of the same product, as shown by mixed m.p., and ultraviolet and infrared spectra.

C. With an excess of acetic anhydride in the absence of pyridine. A suspension of compound 25 (13·3 g, 0·05 mole) in acetic anhydride (190 ml, 2·0 mole) was refluxed for about 5 min until solution resulted. It was allowed to stand at room temperature overnight and was evaporated to dryness on the steam bath *in vacuo* to give a brown solid. Crystallization from 100 ml of water afforded 9·48 g (43·6 per cent) of compound 26, m.p. 187–188°.

D. With hydrogen chloride and an excess of acetic anhydride. One gram of compound 25 was dissolved in acetic acid (20 ml) and 2·5 g of hydrogen chloride was passed through. Acetic anhydride (15 ml) was added to the suspension and the mixture was refluxed until a solution resulted (*ca.* 5 min) and allowed to stand overnight at room temperature. The solution was then evaporated to dryness and the resulting solid was crystallized from acetic acid (5 ml) and ether (20 ml) to give 0·88 g, m.p. 177–179°. An

* Measured by Dr. G. Slomp.

analytical sample was obtained by crystallization from acetic acid–ether; clusters of pale yellow needles, m.p. 181–182·5°. This product was the same as that obtained above, as shown by mixed m.p., and ultraviolet and infrared spectra.

Attempted synthesis of 26 via *the condensation of oxalic acid bishydrazide and 1,3-diacetoxyacetone. A. 1,3-Diacetoxyacetone* was prepared[33] by refluxing a solution of 1,3-dihydroxyacetone and acetic anhydride for 19 h, followed by distillation. It was recrystallized from ether to give colourless needles, m.p. 46–47° (yield, 68 per cent).

B. Oxalic acid bis(1,3-diacetoxyisopropylidene)hydrazide. A suspension of oxalic acid bishydrazide (5·9 g, 0·05 mole), 1,3-diacetoxyacetone (26·1 g, 0·15 mole) and ethanol (600 ml) was refluxed with stirring for 6 h. The small amount of undissolved material was filtered and the filtrate was concentrated to 300 ml and allowed to crystallize; 17·3 g (80·5 per cent) of colourless clusters of needles, m.p. 135–136°, unchanged on recrystallization from ethanol. Ultraviolet spectrum showed $\lambda_{max.}$ 259 (16,400). Infrared spectrum showed NH: 3260; ester C=O: 1742; amide I C=O: 1665: amide II: 1508; C—O/C—N: 1250, 1228, 1190, 1060, 1043.

Anal. Calcd. for $C_{16}H_{22}N_4O_{10}$: C, 44·65; H, 5·15; N, 13·02; CH_3CO, 40.0. Found: C, 44·64; H, 5·30; N, 13·25; CH_3CO, 38·65.

C. Hydrogenolysis to compound 8. The tetraacetoxy compound (8·6 g, 0·02 mole) in ethanol (300 ml) was hydrogenated in the presence of platinum oxide (1 g). Six moles of hydrogen was taken up in about 1 h and the mixture became warm. It was then filtered, and the filtrate was evaporated to about 100 ml and allowed to crystallize; yield 1·45 g (36 per cent), m.p. 178–182°. Recrystallization from ethanol afforded colourless needles, m.p. 188–189° (1·3 g). This product was identical with oxalic acid bis(2-isopropyl)hydrazide (compound 8) by mixture melting point, and ultraviolet and infrared spectra.

Acetylation of compound 25 in the presence of perchloric acid. A solution of perchloric acid (15 ml of 70 per cent perchloric acid and 45 ml of acetic acid) was added during 3 min to a solution of compound 25 (2·66 g, 0·01 mole) in acetic acid (30 ml). Most of the oil which appeared at first dissolved towards the end of addi-

tion. The mixture was stirred for 18 h. Ether (500 ml) was added and the resulting mixture was cooled in ice for 0·5 h. The solid (V as the diperchlorate) was filtered and washed well with ether (very hygroscopic). Ultraviolet spectrum showed $\lambda_{max.}$ 234 (6,350). Infrared spectrum showed NH/OH: 3470, 3180; C=O: 1745, 1730, 1715, 1705, 1655, 1640; amide II: 1560, 1545, 1510, 1500, 1495; C—O: 1275, 1235; ClO_4^-: 1140–1040.

Anal. Calcd. for $C_{16}H_{28}Cl_2N_4O_{18}$: C, 30·25; H, 4·44; N, 8·82; Cl, 11·16. Found: C, 30·52; H, 4·38; N, 9·40; Cl, 11·50.

Oxalic acid bis[2-(2-hydroxy-1-methyl)-propyl]-hydrazide (*27*). *A. Oxalic acid bis[(1-acetyl)-ethylidene]-hydrazide.* A mixture of oxalic acid bishydrazide (23·6 g, 0·2 mole) and diacetyl (68·8 g, 0·8 mole) in ethanol (800 ml) was refluxed with stirring for 4·75 h. The suspension was filtered and the solid washed with ethanol, then with ether. It was crystallized from ethanol and filtered from some insoluble material, to give 29·58 g (59 per cent), m.p. 219–220°, unchanged on further purification. Ultraviolet spectrum showed $\lambda_{max.}$ 263 (27,650). Infrared spectrum showed NH: 3300; C=O: 1706, 1695; C=N: 1608.

Anal. Calcd. for $C_{10}H_{14}N_4O_4$: C, 47·24; H, 5·55; N, 22·04. Found: C, 47·42; H, 5·66; N, 21·56.

B. Compound 27. The bishydrazone (12·7 g, 0·05 mole) was hydrogenated as described in the case of compound 6. The resulting solution was evaporated to dryness and the product crystallized from acetone (50 ml) to give 4 g (31 per cent), melting at 147–149°. It was recrystallized from acetone to give 2·54 g (19·4 per cent), melting at 153·5–155°. The analytical sample melted at 154·5–156·6° (from acetone).

N-*Benzylacrylamide.* Acrylyl chloride was prepared from acrylic acid and benzoyl chloride.[34] Benzylamine (179 g) was added during 40 min to a stirred solution of acrylyl chloride (75·5 g) in benzene (2 l.). The temperature was maintained at 10–15° by means of an ice bath. The resulting suspension was stirred at room temperature for 1 h and then allowed to stand overnight. The suspension was filtered and the precipitate (benzylamine hydrochloride) washed with benzene (2 × 150 ml). The combined benzene solutions were washed in succession with 5 per cent hydrochloric acid solution (3 × 200 ml), water (200 ml), 10 per cent sodium bicarbonate solution (2 × 200 ml), and water

(200 ml) and then with saturated salt solution (2 × 200 ml). The solution was then dried with sodium sulphate, evaporated to about 200 ml and cooled to room temperature. Petroleum ether (200 ml) (30–60°) was added and the resulting crystalline *N*-benzylacrylamide was filtered and washed with petroleum ether; m.p. 65·5–67°, yield, 122 g (90·6 per cent). An analytical sample melted at 67–68° (from benzene–petroleum ether). Reported[35] m.p. 69° (from water). Ultraviolet spectrum showed f 228 (7,050). Infrared spectrum showed NH: 3270; amide I: 1655, 1625; amide II: 1560, 1540; aromatic C=C: 1500; vinyl: 998, 960.

Anal. Calcd. for $C_{10}H_{11}NO$: C, 74·51; H, 6·88; N, 8·69. Found: C, 74·48; H, 6·67; N, 8·97.

Oxalic acid hydrazide 2-[2-(benzylcarbamoyl)-ethyl]hydrazide (28). Solid *N*-benzylacrylamide (8·1 g, 0·05 mole) was added to a solution of hydrazide hydrate (7·5 g, 0·15 mole) in absolute ethanol (25 ml) during 10 min at room temperature. The resulting solution was stirred for 3 h. An earlier experiment had shown that when the above reagents are mixed in a ratio of 1:1, after 1·3 h there is 19 per cent of unreacted *N*-benzylacrylamide, and after 2·3 h, 11 per cent (based on ultraviolet absorption at 228 mμ). A warm solution of methyl hydrazidooxalate[23] (17·7 g, 0·15 mole) in ethanol (200 ml) was added during 5 min to the above solution of crude VI with intermittent ice-cooling. The resulting suspension was stirred at room temperature for 15 min, refluxed for 0·5 h, and allowed to stand overnight. It was then filtered (original filtrate) and the precipitate (18·66 g) washed with ethanol. The solid was purified by boiling with ethanol (1 l.) for 1 h, and the insoluble material (oxalic acid bishydrazide) amounted to 11·5 g, m.p. 244–245°.

The original filtrate was evaporated to *ca.* 300 ml and allowed to crystallize; 5·5 g, m.p. 175–178° (fast). Recrystallization from water (50 ml) afforded clusters of needles; 5·0 g, m.p. 180–183° (slow) which was unchanged on further recrystallization from water.

Oxalic acid bis[2-(benzylcarbamoyl)-ethyl]hydrazide (29). A solution of crude VI was prepared on the same scale as described above for compound 28. A solution of diethyl oxalate (11 g, 0·075 mole) in ethanol (75 ml) was added with occasional cooling during 10 min. The resulting suspension was stirred for 2 h and

then refluxed for 0·5 h. The solid was filtered and washed with ethanol (10·0 g). A second crop amounted to 0·9 g. The two crops were refluxed for 1 h with 1 l. of ethanol. The suspension was filtered hot to give 4·0 g of ethanol-insoluble solid, m.p. 245°, which gave no mixture-melting-point depression with oxalic acid bishydrazide. The filtrate was evaporated and allowed to crystallize; 4·9 g, m.p. 165–185°. It was recrystallized from water (1,050 ml) to give 1·6 g of clusters of needles, m.p. 215–217·5°. Recrystallization from water (1,100 ml) gave 1·5 g, m.p. 216·5–218°.

Syntheses of Various Aliphatic Hydrazides (Table II)

Acetic acid 2-isopropylhydrazide. A. Isopropylidene acethydrazide has been reported to melt at 133°[36,37] and at 139·5–140°.[38] Our sample melted at 138·5–140°.

B. Reduction. A solution of isopropylidene acethydrazide (35·7 g, 0·312 mole) in ethanol (300 ml) and platinum oxide (1·0 g) was hydrogenated at 50°. The resulting solution was evaporated to dryness. Crystallization was achieved from benzene–petroleum ether (30–60°); yield, 24·5 g (68 per cent), m.p. 53·5–55°. The analytical sample melted at 54–56°.

Isobutyric acid 2-isopropylhydrazide. A. Isopropylidene isobutyric acid hydrazide, m.p. 90–91°.[9] Ultraviolet spectrum showed $\lambda_{max.}$ 229 (10,100). Infrared spectrum showed NH: 3180; C=O/C=N: 1665, 1645; amide II: 1545.

B. Reduction. A solution of isobutyric acid isopropylidene hydrazide (32·4 g, 0·228 mole) in ethanol (300 ml) and platinum oxide (1·0 g) was hydrogenated at 50°. The resulting solution was evaporated to dryness and crystallization was achieved with Skellysolve B; yield 25·45 g, (77·5 per cent), m.p. 69–70°, unchanged on further recrystallization.

Malonic acid bis(2-isopropyl)hydrazide. A. Malonic acid bis-isopropylidene bishydrazide, reported m.p. 185°. Our sample melted at 181–183°. Ultraviolet spectrum showed $\lambda_{max.}$ 231 (23,050). Infrared spectrum showed NH: 3180, 3040; C=O/C=N: 1690, 1680, 1670, 1645; amide II: 1560.

B. Reduction. A solution of the bishydrazone (9·0 g, 0·0425 mole) in ethanol (300 ml) and platinum oxide (0·6 g) was hydrogenated at 50°. The resulting solution was evaporated to about

25 ml and diluted with 150 ml of ether to give 7·2 g (80 per cent) of product, m.p. 127–129°. The analytical sample melted at 128–129° (from ethanol–ether).

Succinic acid bis(2-isopropyl)hydrazide. A. Succinic acid bis-isopropylidene-bishydrazide, reported[39] m.p. 200°. In our hands no reaction occurred on refluxing the suspension of succinic acid bishydrazide in acetone for 4 h. The following procedure gave good results: A suspension of the bishydrazide (23·6 g, 0·162 mole), acetone (46·2 g, 0·8 mole) and ethanol (800 ml) was refluxed gently for 3 h. The resulting solution was allowed to crystallize; 18·4 g, m.p. 202–203°. The second crop amounted to 10·25 g, m.p. 203–204°; total yield: 78 per cent. Ultraviolet spectrum showed $\lambda_{max.}$ 232 (25,350). Infrared spectrum showed NH: 3200, 3040; amide I: 1687, 1667; C=N: 1646; amide II: 1548.

B. Reduction. A solution of the bisisopropylidene compound (18·4 g, 0·0815 mole) in ethanol (300 ml) and platinum oxide (1 g) was hydrogenated at 50°. The resulting solution was evaporated to 150 ml and allowed to crystallize; yield 12·8 g (67 per cent), m.p. 158–159·5°, unchanged on further recrystallization.

2,2'-Bisisopropylcarbohydrazide. A. Bisisopropylidene carbohydrazide, reported[40] m.p. 156°. Our sample melted at 161–162°, resolidified and remelted at 210° (efferv.). Ultraviolet spectrum showed $\lambda_{max.}$ 239·5 (20,750). Infrared spectrum showed NH: 3250, 3090; C=O/C=N: 1660, 1640; amide II: 1565, 1560.

Anal. Calcd. for $C_7H_{14}N_4O$: C, 49·39; H, 8·29; N, 32·92. Found: C, 49·36; H, 8·20; N, 33·01.

B. Reduction. The bishydrazone (22·8 g, 0·134 mole) in ethanol (300 ml) and platinum oxide (1 g) were hydrogenated at 50°. Fresh catalyst was added once but only one-half of the theoretical amount of hydrogen was absorbed (the hydrogenation should probably be run in the presence of acid and at room temperature since the bishydrazone undergoes a change on heating in solution). The solution was evaporated to dryness and allowed to crystallize from acetone to give 5·16 g of starting material. The filtrate was concentrated and allowed to crystallize; needles, m.p. 123–129°. Recrystallization from acetone raised the m.p. to 141–142° (1·45 g). An analytical sample melted at 142–143°.

Syntheses of Aromatic Hydrazides (Table III)

N^2-*Isopropylsalicylic acid hydrazide. Isopropylidene salicylic acid hydrazide* (reported m.p. 229–230°[41]) (18·7 g, 0·0975 mole) in ethanol (300 ml) and 1 g of platinum oxide was hydrogenated at 50°. The solution was filtered and evaporated to dryness and the resulting colourless solid was dissolved in ether (250 ml) and allowed to crystallize from 75 ml; yield, 6·8 g, m.p. 101–110°. Fractional crystallization from ether afforded 1·84 g, m.p. 113–115°, unchanged on recrystallization. A second crop amounted to 4·02 g, m.p. 113–114°, a third crop, 2·6 g, m.p. 112–114·5.°

The crude mixture (2 g) from another hydrogenation run was dissolved in acetone (1 ml) and benzene (10 ml) and chromatographed on Florisil (200 g). Elution with 6 per cent acetone–Skellysolve B (4,800 ml) gave 827·9 mg of solid which was crystallized from ether and afforded 0·7 g of N^2-isopropylsalicylic acid hydrazide, m.p. 113–115°. Elution with 9–20 per cent acetone–Skellysolve B gave 211·6 mg which was discarded. Elution with 30 per cent acetone–Skellysolve B gave 126·2 mg (m.p. 92–96°) which was crystallized from ether to give long needles of 2-hydroxycyclohexanecarboxylic acid N^2-isopropylhydrazide, m.p. 97–99°. Ultraviolet spectrum showed end absorption. Infrared spectrum showed OH/NH: 3280, 3240; C=O: 1640 sh, 1630; amide II: 1545 sh, 1537.

Anal. Calcd. for $C_{10}H_{20}N_2O_2$: C, 59·97; H, 10·07; N, 13·99 Found: C, 59·80; H, 10·21; N, 14·33.

N^2-*Isopropyl-3,4,5-trimethoxybenzoic acid hydrazide. A. Isopropylidene 3,4,5-trimethoxybenzhydrazide.* A solution of 3,4,5-trimethoxybenzhydrazide (22·6 g, 0·1 mole) (see Table III) in acetone (500 ml) was refluxed for 2·5 h. It was evaporated to dryness and the residue was crystallized from ethanol–ether; yield 19·05 g, m.p. 139–140°, unchanged on further purification. A second crop amounted to 4·82 g, m.p. 134–135°; yield, 89·5 per cent. Ultraviolet spectrum showed $\lambda_{max.}$ 214 (34,500); 268 (13,400). Infrared spectrum showed NH: 3200; C=O: 1645 sh, 1630; C=C: 1585, 1503; amide II: 1530.

Anal. Calcd. for $C_{13}H_{18}N_2O_4$: C, 58·63; H, 6·81; N, 10·52. Found: C, 58·82; H, 7·22; N, 10·54.

B. Reduction. The isopropylidene compound (10·38 g, 0·0388 mole) in ethanol (300 ml) and platinum oxide (0·4 g) was hydrogenated. The resulting solution was evaporated and allowed to crystallize; yield, 7·93 g (76 per cent), m.p. 146·5–148°. The analytical sample melted at 147·5–148·5°.

Synthesis of 1-(1-hydroxymethyl-2-hydroxy)-ethyl-2-(3,4,5-trimethoxy)-benzhydrazide. A. 1,3-Dihydroxyisopropylidene-3,4,5-trimethoxybenzhydrazide. A mixture of 3,4,5-trimethoxybenzhydrazide (22·6 g, 0·1 mole) (see Table III) and 1,3-dihydroxyacetone (freshly distilled) (9·9 g, 0·11 mole) in ethanol (260 ml) was refluxed under nitrogen with stirring for 1 h. An aliquot corresponding to 0·01 mole was then evaporated to dryness and the residual oil crystallized from ethanol; yield 1·85 g, m.p. 139–141°. Recrystallization from ethanol afforded pale yellow needles, m.p. 141·5–142·5°, unchanged on further recrystallization. Ultraviolet spectrum showed $\lambda_{max.}$ 218 (31,300); 274 (16,550). Infrared spectrum showed OH/NH: 3500, 3260 sh, 3160; =CH: 3010; C=O/C=N: 1663, 1647; C=C: 1590; amide II: 1550 sh, 1540; C—O/C—N: 1340, 1240, 1120, 1040, 1008; ring: 855, 763, 746, 730.

Anal. Calcd. for $C_{13}H_{18}N_2O_6$: C, 52·34; H, 6·08; N, 9·39. Found: C, 52·39; H, 5·58; N, 9·54.

B. Reduction. The remainder of the above solution (corresponding to 0·09 mole of starting material) was hydrogenated in the presence of platinum oxide (1 g). The hydrogenation was interrupted when 0·09 mole of hydrogen was taken up (2·5 h). The solution was evaporated to dryness at 25°, the oily solid residue was crystallized from ethanol (20 ml) and ether (70 ml); yield 15·9 g, m.p. 82–90°. Recrystallization from ethanol–ether gave 10·4 g, m.p. 95–98° (efferv.). Further crystallization from ethanol–ether raised the m.p. to 111–112·5° (efferv.), unchanged on additional purification.

N^2-*(1-Hydroxymethyl-2-hydroxy)ethylisonicotinhydrazide. A. 1,3-Dihydroxyisopropylidene isonicotinhydrazide.* A mixture of isonicotinhydrazide (13·7 g, 0·1 mole), 1,3-dihydroxyacetone (18 g, 0·2 mole) and ethanol (360 ml) was refluxed for 1·1 h under nitrogen. The resulting brown suspension was filtered and the yellow solid (9·5 g) was crystallized from ethanol (400 ml); yield 6·0 g, m.p. 194–195 (d.). Prolonged heating of the solution

should be avoided since it tends to become dark brown and one cannot obtain second crops even during recrystallization of pure material. A sample was recrystallized for analysis from ethanol; clusters of prisms, m.p. 146–146·5° (d.). Ultraviolet spectrum showed $\lambda_{max.}$ 266 (10,700). Infrared spectrum showed OH/NH: 3240, 3160; C=O: 1685; C=N: 1652; amide II: 1629.

Anal. Calcd. for $C_9H_{11}N_3O_3$: C, 51·67; H, 5·30; N, 20·09. Found: C, 51·63; H, 5·38; N, 19·69.

B. Reduction. A suspension of the 1,3-dihydroxyisopropylidene compound (5·7 g, 0·0272 mole) in ethanol (200 ml) was hydrogenated in the presence of platinum oxide (0·5 g). The reaction was interrupted after 1·25 h when one equivalent of hydrogen was absorbed. The resulting solution was evaporated to dryness at $< 50°$ and the brown solid residue was crystallized from methanol–benzene to give 40 g (70 per cent) of pale yellow solid, m.p. 124–127° (fast). No second crop could be obtained. Recrystallization from benzene–methanol afforded a sample melting at 123–124°.

N^2-*Isopropyl-3-hydroxy-2-naphthoic acid hydrazide. A. Isopropylidene 3-hydroxy-2-naphthoic acid hydrazide.* A suspension of 3-hydroxy-2-naphthoic acid hydrazide (10 g, 0·0495 mole) (see Table III) in acetone (500 ml) was refluxed for 6·5 h. The resulting suspension was cooled and filtered to give 9·9 g (83 per cent) of yellow needles, m.p. 241–242°, unchanged on recrystallization from ethanol. Ultraviolet spectrum showed f 216 (34,250); 238 (68,250); 242 (69,300); 250 (63,500); f 264 (38,600); f 272 (29,350); 286·5 (24,700); 298 (23,050); 350 (3,000). Infrared spectrum showed OH/NH: 3240, 3000; C=O: 1645; C=N: 1625; amide II: 1552.

Anal. Calcd. for $C_{14}H_{14}N_2O_2$: C, 69·40; H, 5·83; N, 11·56. Found: C, 69·31; H, 5·90; N, 11·47.

B. Reduction. The isopropylidene derivative (9·6 g, 0·0397 mole) in ethanol (300 ml) and 0·5 g of platinum oxide were hydrogenated at 50°. The resulting yellow solution was concentrated to *ca.* 25 ml and allowed to crystallize; the yield of clusters of pale brown needles was 3·9 g, m.p. 146–147°. A second crop amounted to 2·5 g, m.p. 149–152°. The analytical sample melted at 149·5–151°.

N^2-*Isopropylindole-3-acethydrazide. A. Indole-3-acethydrazide*

was prepared from ethyl 3-indoleacetate[42] as described previously[22] (reported m.p. 138–139°). Our sample melted at 140–142° and was obtained in 32 per cent yield by refluxing the ester (51·5 g), hydrazine hydrate (19·3 g) and ethanol (510 ml) for 6 h.

B. Isopropylidene indole-3-acethydrazide. A solution of the hydrazide (14·28 g, 0·0815 mole) in acetone (250 ml) was refluxed for 2 h. The solvent was evaporated until crystallization started; colourless needles, 12·6 g, m.p. 168·5–169·5°, unchanged on recrystallization. A second crop amounted to 1·21 g, m.p. 167–168·5°; yield, 74 per cent. Ultraviolet spectrum showed $\lambda_{max.}$ 220 (42,700); 273 (6,300); 280 (6,450); 290 (5,500). Infrared spectrum showed NH: 3410; C=O: 1663; C=N: 1618.

Anal. Calcd. for $C_{13}H_{15}N_3O$: C, 68·10; H, 6·59; N, 18·33. Found: C, 67·93; H, 6·54; N, 18·57.

C. Reduction. The isopropylidene derivative (13·5 g, 0·059 mole) in ethanol (300 ml) and platinum oxide (0·5 g) was hydrogenated at 50°. The resulting yellow solution was evaporated to dryness and ether (200 ml) was added to give a solid, melting at 126·5–130°. Crystallization from water raised the m.p. to 130–131° (6·3 g). The analytical sample melted at 133–134° (from water).

Summary. A number of aliphatic and aromatic hydrazides were synthesized and tested as inhibitors of monoamine oxidase. The physical and spectral properties and the *in vitro* results of these compounds are compiled in Tables I, II and III. The *in vivo* results of several derivatives of oxalic acid bishydrazides are compiled in Tables IV and V.

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