Relationship between CNS Depressant and Enzyme Inhibitory Properties of Substituted Quinazolone 1,3,4-Oxadiazoles

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Abstract □ Substituted quinazolone 1,3,4-oxadiazoles were synthesized and evaluated for their MAO and acetylcholinesterase inhibitory activity. These compounds were also screened for their anticonvulsant activity, reserpine reversal response, and analgesic activity. Anticonvulsant activity of these compounds against pentylenetetrazol-induced seizures was related to some extent to their MAO and acetylcholinesterase inhibitory properties.

Keyphrases □ Quinazolone 1,3,4-oxadiazoles—synthesis, anticonvulsant and analgesic activity, relationship to MAO and acetylcholinesterase inhibition □ 1,3,4-Oxadiazoles, quinazolone—synthesis, anticonvulsant and analgesic activity, relationship to MAO and acetylcholinesterase inhibition □ Anticonvulsant activity—synthesis and evaluation of quinazolone 1,3,4-oxadiazoles □ MAO inhibitors—synthesis and evaluation of quinazolone 1,3,4-oxadiazoles □ Acetylcholinesterase inhibitors—synthesis and evaluation of quinazolone 1,3,4-oxadiazoles

Anticonvulsant (1) and hypnotic (2) properties exhibited by quinazolones and the specific inhibition of MAO [E.C. 1.4.3.4 monoamine O_2 oxido reductase-(deaminating)] by 1-isonicotinoyl-2-isopropylhydrazine (3) prompted the synthesis of substituted quinazolone hydrazides as MAO inhibitors (4, 5). Furthermore, the ability of substituted 1,3,4-oxadiazoles to exhibit analgesic (6, 7), anti-inflammatory, antipyretic (8, 9), muscle relaxant (9), and CNS depressant activities (10, 11) led us to synthesize substituted quinazolone 1,3,4-oxadiazoles. In the present study, attempts were made to correlate pharmacological properties exhibited by these compounds with their ability to inhibit MAO and acetylcholinesterase.

CHEMISTRY

The various substituted quinazolone 1,3,4-oxadiazoles were synthesized by the route outlined in Scheme I. Acetanthranils (II) were synthesized by refluxing 1 mole of the appropriate anthranilic acid (I) with 2 moles of acetic anhydride. Quinazolone esters (IV) were synthesized by heating equimolar quantities of appropriate acetanthranils (II) and ethyl glycine ester (III). Quinazolone hydrazides (V) were synthesized by refluxing a mixture of quinazolone esters (IV) and hydrazine hydrate. Substituted quinazolone thiosemicarbazides (VI) were prepared by refluxing the appropriate hydrazide (V) with a suitable arylisothiocyanate in ethanol. These quinazolone thiosemicarbazides were cyclized into substituted quinazolone 1,3,4-oxadiazoles (VII).

EXPERIMENTAL

Melting points were taken in open capillary tubes and are uncorrected.

Anthranilic Acids (I)—The method of Atkinson and Milton (12) was used for the synthesis of 5-chloro- and 3,5-dichloroanthranilic acids. 5-Bromoanthranilic acid was prepared by the method of Wheeler and Oats (13), while 5-iodoanthranilic acid was synthesized following the method of Klemme and Hunter (14).

Acetanthranil (II)—Appropriate anthranilic acids (1 mole) were refluxed with acetic anhydride (2 moles) for 1 hr. After excess acetic anhydride was distilled off, the acetanthranil that separated out as a solid mass was dried quickly and used without further purification.

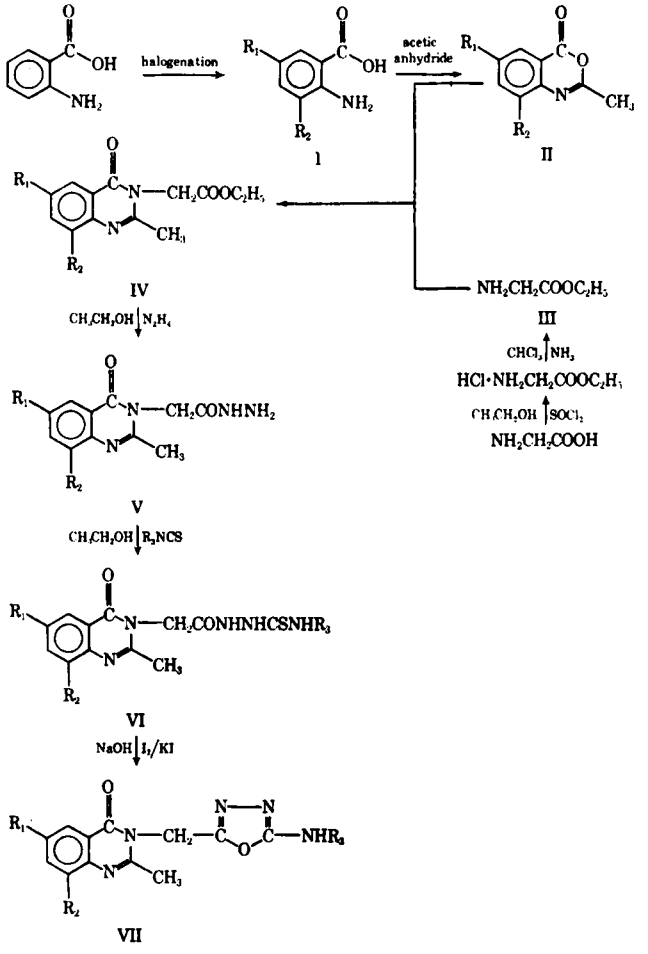
Ethyl Glycine Ester (III)—This ester was prepared by following the method of Kuprozewski and Sokolowaska (15).

Quinazolone Esters (IV)—Equimolar quantities (1 mole) of the appropriatea cetanthranil and ethyl glycine ester were heated directly to yield the desired esters, which were recrystallized from ethanol (Table I).

Quinazolone Hydrazides (V)—A mixture of the appropriate quinazolone ester (0.1 mole) and hydrazine hydrate (98%) (0.12 mole) was refluxed in absolute ethanol for 6–8 hr. On removing the excess solvent, the quinazolone hydrazides that separated out were filtered and recrystallized from ethanol (Table I).

Quinazolone Thiosemicarbazides (VI)—Equimolar quantities of hydrazides and arylisothiocyanate (0.01 mole) were refluxed in ethanol for 3–4 hr. The solution was cooled to obtain corresponding thiosemicarbazides, which were recrystallized from suitable solvents (Table II).

Quinazolone 1,3,4-Oxadiazoles (VII)—The quinazolone thiosemicarbazides were cyclized into quinazolone 1,3,4-oxadiazoles by following the method of Silberg and Cosma (16). To a cold ethanolic suspension of an appropriate thiosemicarbazide (0.01 mole) was added 5 ml. of 4 *N* NaOH solution, followed by 5% solution of iodine in potassium iodide which was gradually added to the clear solution with stirring until the color of iodine persisted at room



Scheme I

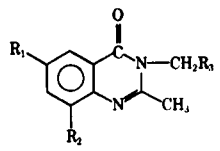


Table I—Substituted Quinazolone Esters and Hydrazides

Compound Number	R_1	R_2	R_3	Melting Point	Yield, %	Molecular Formula (Molecular Weight)	Analysis, % Nitrogen Calc.	Analysis, % Nitrogen Found
1	H	H	$COOC_2H_5$	130–131°	60	$C_{13}H_{14}N_2O_3$	11.38	11.21
2	H	H	$CONHNH_2$	261°	55	$C_{11}H_{12}N_4O_2$	24.13	23.80
3	Cl	H	$COOC_2H_5$	80°	60	$C_{13}H_{13}ClN_2O_3$	9.97	9.53
4	Cl	H	$CONHNH_2$	260°	50	$C_{11}H_{11}N_4O_2$	21.01	20.61
5	Br	H	$COOC_2H_5$	81°	70	$C_{13}H_{13}BrN_2O_3$	8.61	8.24
6	Br	H	$CONHNH_2$	248°	60	$C_{11}H_{11}BrN_4O_2$	18.00	17.60
7	I	H	$COOC_2H_5$	108°	70	$C_{13}H_{13}IN_2O_3$	7.52	7.31
8	I	H	$CONHNH_2$	265°	50	$C_{11}H_{11}IN_4O_2$	18.43	18.21
9	Cl	Cl	$COOC_2H_5$	160°	60	$C_{13}H_{12}Cl_2N_2O_3$	8.88	8.56
10	Cl	Cl	$CONHNH_2$	275°	55	$C_{11}H_{10}Cl_2N_4O_2$	18.60	18.32
11	Br	Br	$COOC_2H_5$	175°	70	$C_{13}H_{12}Br_2N_2O_3$	6.93	6.72
12	Br	Br	$CONHNH_2$	285°	50	$C_{11}H_{10}Br_2N_4O_2$	16.92	16.75

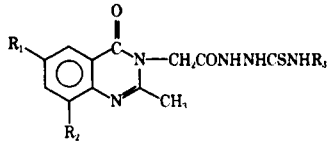


Table II—Substituted Quinazolone Thiosemicarbazides

Compound Number	R_1	R_2	R_3	Crystallization Solvent	Yield, %	Melting Point	Molecular Formula		Analysis, % Calc.	Analysis, % Found
13	H	H	*m*-$CH_3C_6H_4$	Ethanol	60	217–218°	$C_{19}H_{19}N_5O_2S$	C	59.84	59.97
								H	4.98	5.09
								N	18.37	17.89
14	H	H	*p*-$CH_3C_6H_4$	Ethanol	65	226–228°	$C_{19}H_{19}N_5O_2S$	C	59.84	59.87
								H	4.98	5.12
								N	18.37	17.76
15	H	H	*p*-$CH_3OC_6H_4$	Ethanol	70	219–220°	$C_{19}H_{19}N_5O_3S$	C	57.42	57.37
								H	4.78	5.14
								N	17.63	17.94
16	Br	H	*m*-$CH_3C_6H_4$	Ethanol	55	234–235°	$C_{19}H_{18}BrN_5O_2S$	C	49.56	49.88
								H	3.91	4.12
								N	15.22	14.95
17	Br	H	*p*-$CH_3OC_6H_4$	Ethanol	60	235–237°	$C_{19}H_{18}BrN_5O_3S$	C	47.90	48.47
								H	3.78	4.12
								N	14.61	14.42
18	Cl	Cl	*m*-$CH_3C_6H_4$	Acetic acid	70	290–292° dec.	$C_{19}H_{17}Cl_2N_5O_2S$	C	50.66	50.35
								H	3.77	3.87
								N	15.56	15.39
19	Cl	Cl	*p*-$CH_3C_6H_4$	Ethanol	65	260–265° dec.	$C_{19}H_{17}Cl_2N_5O_2S$	C	50.66	50.42
								H	4.77	4.33
								N	15.56	15.32
20	Cl	Cl	*p*-$CH_3OC_6H_4$	Acetic acid–ether	70	284–286°	$C_{19}H_{17}Cl_2N_5O_3S$	C	48.92	48.65
								H	3.64	4.12
								N	15.02	14.95

temperature. The contents were then refluxed on a steam bath, and more iodine solution was added until a slight excess of iodine remained. The reaction mixture was cooled and poured into 500 ml. of ice-cold water. The solid masses that separated out were filtered, washed with water and then with carbon disulfide, and recrystallized from suitable solvents (Table III).

BIOCHEMICAL STUDIES

***In Vitro* Determination of MAO Activity**—MAO activity of rat liver homogenate was determined spectrophotofluorometrically using kynuramine as the substrate (17). The enzyme activity was determined by measuring the 4-hydroxyquinoline formed during oxidative deamination of kynuramine. The reaction mixture, in a final concentration of 3 ml., consisted of 83 m*M* phosphate buffer (pH 7.4) and 1 ml. of the liver homogenate (10% w/v). Water, kynuramine, and the test compounds were added to adjust the final volume to 3.0 ml. The reaction was conducted in test tubes, and incubations were carried out at 37° for 30 min. After incubation, 2 ml. of 10% trichloroacetic acid (w/v) was added to each test tube and the precipitated proteins were removed by centrifugation. Suitable aliquots of the supernate, taken in 1 *N* NaOH solution, were assayed for 4-hydroxyquinoline fluorometrically in a spectrophotofluorometer[1], using activating light of 310 nm. and measuring fluorescence at the maximum of 380 nm. An increase in absorbance provided a direct measurement of 4-hydroxyquinoline as an index of kynuramine utilization and, consequently, of MAO activity. A decrease in kynuramine utilization in experiments containing the test compounds was used to calculate the degree of MAO inhibition.

***In Vitro* Determination of Acetylcholinesterase Activity**—Acetylcholinesterase activity was determined colorimetrically, using acetylthiocholine iodide (0.015 *M*) as the substrate, according to the method of McOskar and Daniel (18). The reaction mixture in final concentration consisted of 43 m*M* tromethamine buffer (pH 7.4), 350 m*M* sodium chloride, and 0.3 ml. brain homogenate (10% w/v).

[1] Aminco-Bowman.

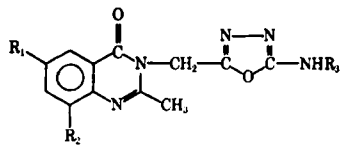


Table III—Substituted Quinazolone 1,3,4-Oxadiazoles

Compound Number	R_1	R_2	R_3	Yield, %	Melting Point	Molecular Formula	Analysis, % Calc.	Found
21	H	H	*m*-$CH_3C_6H_4$	50	180–182°	$C_{19}H_{17}N_5O_2$	C 65.70	65.61
							H 4.89	5.44
							N 20.17	19.80
22	H	H	*p*-$CH_3C_6H_4$	45	210–211°	$C_{19}H_{17}N_5O_2$	C 65.70	65.55
							H 4.89	4.84
							N 20.97	19.69
23	H	H	*p*-$CH_3OC_6H_4$	50	207–209°	$C_{19}H_{17}N_5O_3$	C 62.55	62.75
							H 4.68	4.89
							N 19.29	18.80
24	Br	H	*m*-$CH_3C_6H_4$	45	123–129°	$C_{19}H_{16}BrN_5O_2$	C 53.51	53.64
							H 3.75	4.08
							N 16.43	15.97
25	Br	H	*p*-$CH_3OC_6H_4$	55	222–224°	$C_{19}H_{16}BrN_5O_3$	C 51.58	51.25
							H 3.62	3.84
							N 15.84	15.35
26	Cl	Cl	*m*-$CH_3C_6H_4$	45	203°	$C_{19}H_{15}Cl_2N_5O_2$	C 54.80	54.56
							H 3.60	4.10
							N 16.83	16.35
27	Cl	Cl	*p*-$CH_3C_6H_4$	50	249–250°	$C_{19}H_{15}Cl_2N_5O_2$	C 54.80	55.00
							H 3.60	3.98
							N 16.83	16.31
28	Cl	Cl	*p*-$CH_3OC_6H_4$	40	213–215°	$C_{19}H_{15}Cl_2N_5O_3$	C 52.77	52.71
							H 3.47	3.64
							N 16.20	15.76

Water and acetylthiocholine were added to adjust the final volume to 2.0 ml. The reaction mixture, with or without inhibitor, was incubated at constant temperature (37°) for 10 min. prior to the addition of acetylthiocholine. The incubation was continued for 10 min. more subsequent to the addition of the substrate. The reaction was stopped by the addition of 0.5 ml. of 25% (w/v) trichloroacetic acid, and the resultant solution was centrifuged for 5 min. at 500× *g*. An aliquot of the clear supernate was withdrawn, and the enzymatically formed thiocholine content was determined colorimetrically. Each assay was done in triplicate, and tissue and substrate blanks were substracted to give the actual value for the hydrolysis of acetylthiocholine. Results are expressed as changes in absorbance/100 mg. fresh tissue/10 min.

PHARMACOLOGICAL STUDIES

Toxicity—The approximate 50% lethal dose (LD_{50}) was determined in albino mice by the method of Smith (19). The dose of each compound was taken as one-fifth of its observed LD_{50} in an attempt to evaluate it in its effective therapeutic range. The test compounds were administered to each group of 10 mice orally in 5% gum acacia.

Reserpine Reversal Test for *In Vivo* Determination of MAO Inhibitory Activity—Reserpine was administered intraperitoneally in a dose of 5 mg./kg. after 3 hr. In the control group of mice, without pretreatment of drugs, reserpine always produced sedation, ptosis, and miosis. In animals treated with phenelzine and pargyline, the well-known MAO inhibitors, administration of reserpine caused excitation, piloerection, mydriasis, and increased motor activity. Failure to produce the reserpine reversal response by the test compounds indicated that the compound was inactive in producing *in vivo* MAO inhibition.

Anticonvulsant Activity—The anticonvulsant property was determined in albino mice of either sex, weighing between 20 and 30 g., divided into groups of 10; the group weights were kept equal as far as possible. One group of 10 animals was used as the control. The test compounds were suspended in 5% aqueous gum acacia and were fed orally 3 hr. prior to subcutaneous injection of pentylenetetrazol[2], 80 mg./kg., under the loose skin of the back. This dose of pentylenetetrazol caused convulsions in almost all of the normal control group of animals. The animals were observed for 60 min. for the occurrence of seizures; an episode of clonic spasm persisting for at least 5 sec. was considered a threshold convulsion. Animals not exhibiting even a threshold convulsion during 60 min. were considered protected. The number of animals protected in each group were recorded. Transient intermittent jerks or tremulousness were not taken into account. Phenelzine and pargyline were used for comparison.

Analgesic Activity—*Mouse Tail-Pinch Test*—Morphine-like analgesic activity was tested by the mouse tail-pinch method (20). An artery clip, with branches enclosed in a thin rubber tube, was applied to the root of the tail of a mouse for 30 sec. The animals made continuous attempts to remove the noxious stimulus by biting the clips. Mice were administered test compounds by the intragastric route 3–4 hr. prior to the application of the artery clips. The insensitivity to the noxious stimulus was taken as the positive response, and the results were expressed as percentage of mice showing analgesic activity. Morphine hydrochloride was used as a reference standard for comparing the effective analgesic property of these compounds.

Writhing Test—Aspirin-like analgesic activity of the test compounds was investigated by their ability to protect a painful writhing syndrome. This syndrome is characterized by abdominal torsion, drawing up of hind limbs to the abdominal wall, marked contraction of the abdominal area, and periodic arching of the back to rub the abdominal wall on the glazed surface on which the mouse was kept. It was consistently produced by intraperitoneal injection of 2 mcg. aconitine (100% effective dose) per mouse weighing 20–25 g. The test compounds were given 3 hr. before the induction of the writhing test. The typical response appeared within 5 min. after injection of aconitine and persisted up to 15–20 min. The mice were observed for 30 min., and results are expressed as percent of mice showing protection. Aspirin was taken as a standard reference drug for comparison.

RESULTS AND DISCUSSION

The test compounds exhibited low *in vitro* MAO inhibitory activity (Table IV) as compared to the 78% inhibition produced by phenelzine and pargyline when used at final concentrations of 1×10^{-5} and 1×10^{-6} *M*, respectively.

[2] Metrazol.

Table IV—Biochemical and Pharmacological Studies of Substituted Quinazolones, Substituted Quinazolone Hydrazides, Thiosemicarbazides, and 1,3,4-Oxadiazoles

Compound Number[a]	MAO Inhibition, %		Antiacetylcholinesterase Activity,	LD_{50},	Dose,	Anticonvulsant Activity Protection,	Reserpine Reversal Response	Analgesic Activity	
	6×10^{-4} M	3×10^{-4} M	3×10^{-4} M	mg./kg.	mg./kg.	%		Tail-Pinch	Aconitine Writhing Response Test, %
1	Nil	—	40.53	800	160	10	(—)	Nil	20
2	Nil	—	Nil	800	160	10	(—)	Nil	Nil
3	16.52	—	10.53	800	160	20	(—)	Nil	Nil
4	22.64	—	Nil	800	160	20	(—)	Nil	20
5	Nil	—	26.32	1600	320	10	(—)	Nil	Nil
6	Nil	—	15.79	800	160	20	(—)	Nil	20
7	25.80	—	44.73	400	80	20	(—)	Nil	20
8	19.42	—	23.68	400	80	30	Slight stimulation	Nil	20
9	34.88	—	39.47	1600	320	30	Slight activity	Nil	20
10	38.70	—	21.05	1600	320	50	(—)	Nil	Nil
11	25.80	—	26.32	1600	320	30	(—)	Nil	Nil
12	47.81	—	21.05	400	80	40	Slight stimulation	Nil	80
13	—	77.26	68.42	400	80	50	(—)	Nil	40
14	—	72.29	44.73	1600	320	50	(—)	Nil	Nil
15	—	78.45	47.37	800	160	60	Slight stimulation	Nil	40
16	—	82.22	63.15	1600	320	50	(—)	Nil	20
17	—	80.09	42.10	400	80	50	Slight activity	Nil	Nil
18	—	69.55	57.89	1600	320	70	Slight stimulation	Nil	Nil
19	—	69.38	42.10	400	80	50	(—)	Nil	40
20	—	73.28	42.10	1600	320	50	Slight stimulation	Nil	20
21	—	41.91	21.05	800	160	30	(—)	Nil	20
22	—	56.89	57.89	1600	320	60	(—)	Nil	20
23	—	56.28	21.05	1600	320	20	Slight stimulation	Nil	Nil
24	—	21.12	52.63	400	80	40	(—)	Nil	Nil
25	—	45.10	44.73	800	160	50	Slight activity	Nil	Nil
26	—	23.21	57.89	400	80	50	(—)	Nil	Nil
27	—	Nil	67.89	1600	320	30	(—)	Nil	40
28	—	36.20	52.63	1600	320	70	Slight stimulation	Nil	20

[a] Compound numbers are as recorded in Tables I, II, and III.

As is evident from Table IV, acetylcholinesterase inhibitory properties of substituted quinazolone hydrazides were lower than those of their corresponding esters. Attachment of a halogen substituent at position 6 or 8 of the quinazolone nucleus in no way reflected any basis for their structure–activity relationship with respect to their ability to inhibit MAO and acetylcholinesterase. However, the anticonvulsant activity exhibited by quinazolone hydrazides, as reflected by the protection observed against pentylenetetrazol-induced convulsions, was found to be more than that observed with the corresponding quinazolone esters. The anticonvulsant activity of phenelzine and pargyline, used for comparison, was found to be 50% at a dose of 30 mg./kg. under similar experimental conditions. Most of the quinazolone esters and quinazolone hydrazides were devoid of reserpine reversal response. These results thus indicated their weak *in vivo* MAO inhibitory activity. Furthermore, a number of compounds in the group of quinazolone thiosemicarbazides and 1,3,4-oxadiazoles exhibited slight stimulation on administration of reserpine which, however, was found to have no relationship with their MAO inhibitory potency. Compounds 16, 19, and 22 showed over 50% inhibition but were devoid of reserpine reversal response, while slight stimulation on administration of reserpine was observed with compounds possessing a low MAO inhibitory property (Compounds 7, 8, 12, and 28).

The results in Table IV show that a large number of compounds provided protection against aconitine-induced writhing response where 2-methyl-6,8-dibromo-3-(acetylhydrazino)-4-quinazolone (Compound 12) exhibited appreciable analgesic activity of 80%. None of these quinazolone esters, hydrazides, thiosemicarbazides, and 1,3,4-oxadiazoles gave a positive response when tested for their morphine-like analgesic activity by the tail-pinch method; the standard reference drug, aspirin, showed 80% protection when administered at a dose of 40 mg./kg. Such analgesic activity warrants further detailed investigation for the possible usefulness of this compound as a nonsteroidal, anti-inflammatory agent. In addition, the high LD_{50} values observed with these compounds reflected their low toxicity.

Conversion of the quinazolone hydrazides into the corresponding quinazolone thiosemicarbazides resulted in increased MAO and acetylcholinesterase inhibitory potency. Significant MAO inhibition, ranging from 69.38 to 82.22%, was observed with these substituted quinazolone thiosemicarbazides even at half of their concentration of 3×10^{-4} M as compared to 6×10^{-4} M used for substituted quinazolone esters and hydrazides (Table IV). (Compare Compounds 1 and 2 with 13, 14, and 15; 5 and 6 with 16 and 17; and 9 and 10 with 18, 19, and 20.) Greater inactivation of MAO and acetylcholinesterase was similarly observed with substituted quinazolone 1,3,4-oxadiazole as compared to the corresponding quinazolone esters and hydrazides which, however, were less potent than the quinazolone thiosemicarbazides. Although these results indicated the greater sensitivity of the quinazolone thiosemicarbazides to inhibit MAO and acetylcholinesterase than the corresponding substituted quinazolone hydrazides and quinazolone 1,3,4-oxadiazoles, no clearcut relationship of their enzyme inhibitory effects as a function of their chemical structure could be observed. These results also failed to provide similarity of substituted quinazolone esters, hydrazides thiosemicarbazides, and 1,3,4-oxadiazoles in their inhibitory effects on MAO, a dehydrogenase in which flavins seem to be implicated, and acetylcholinesterase, a hydrolase without any cofactor requirements.

The anticonvulsant activity exhibited by these compounds, by affording protection against pentylenetetrazol-induced seizures, was found to be related with their MAO and acetylcholinesterase inhibitory properties. These studies reflected a greater anticonvulsant property, ranging from 50 to 70% of substituted quinazolone thiosemicarbazides which were also found to cause greater inhibition of MAO and acetylcholinesterase. In the present study, enzyme inhibitory effects and anticonvulsant activity of these compounds were in the order of: thiosemicarbazides > oxadiazoles > hydrazides > esters.

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Solubility and Dissolution of Triamcinolone Acetonide

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Abstract □ Available data on the solubility of triamcinolone acetonide in aqueous media are neither complete nor in agreement. Solubility of the steroid in distilled water and in potassium chloride solutions of varying ionic strength was determined at 28, 37, and 50°. Dissolution rates of the steroid in the aqueous media were determined at 28° *via* a rotating-disk method. Triamcinolone acetonide solubility in distilled water ranged from 21 mcg. ml.$^{-1}$ at 28° to 33.6 mcg. ml.$^{-1}$ at 50°. The differential heat of solution was 4207 cal. mole^{-1}. The intrinsic dissolution rate constant at the maximum agitation intensity employed was 4.51×10^{-3} hr.$^{-1}$ cm.$^{-2}$. Solubilities and dissolution rates were markedly lower in potassium chloride solutions. "Salting-out" coefficients were calculated, for the effect of potassium chloride on triamcinolone acetonide solubility, using the empirical Setschenow equation.

Keyphrases □ Triamcinolone acetonide—solubility and dissolution in water and potassium chloride solutions □ Solubility, triamcinolone acetonide—water and potassium chloride solutions, Setschenow salting-out coefficients □ Dissolution rates, triamcinolone acetonide—water and potassium chloride solutions

Lange and Amudson (1), using a gravimetric procedure, determined the aqueous solubility of triamcinolone acetonide to be 26.4 mg./100 ml. at 25°. Malkinson and Kirschenbaum (2), in their study of the percutaneous absorption of triamcinolone acetonide, noted that the steroid had an aqueous solubility of 1 mg./100 ml., although neither the temperature of the system nor the method used in determining solubility was specified. Florey (3) recently reported solubilities for the compound in water as well as isotonic saline (pH 7) at 23 and 37° as 0.004 ± 0.002% (40 mcg./ml.). The solubility data available thus far are neither adequate nor in agreement. No data have been reported on the effect of ionic strength on the steroid's solubility, nor are dissolution rate data available. Since the solubility and dissolution of the steroid can ultimately affect its release from matrixes in which it is suspended, these physicochemical properties of triamcinolone acetonide were evaluated.

MATERIALS AND METHODS

Materials—Hydrochloric acid[1], potassium chloride[1], recrystallized blue tetrazolium[1], tetramethylammonium hydroxide (10% in water)[2], and triamcinolone acetonide[3] were used as received.

One potential source of error—the polymorphic form of the steroid—was eliminated following differential thermal analyses of the three lots of steroid received. No marked differences in the thermograms obtained[4] were noted. It could thus be concluded that the steroid employed was of a single polymorphic form.

Reagents—Blue tetrazolium solution (0.2% w/v) was prepared by accurately weighing 100 mg. of blue tetrazolium, suspending the material in 35 ml. of alcohol, and agitating the suspension at 45° until dissolution was complete. Following the attainment of ambient thermal equilibrium, the solution volume was adjusted to 50 ml. with alcohol. The solution was freshly prepared before use. Tetramethylammonium hydroxide solution (1% v/v) was prepared from the commercial 10% solution by accurately transferring 10 ml. of the concentrate to a volumetric flask and adjusting the volume to 100 ml. with alcohol. Hydrochloric acid (1 *N*) was prepared by diluting 12.5 ml. of concentrated acid to 100 ml. with distilled water.

[1] Fisher Scientific Co., Fair Lawn, N. J.
[2] Eastman Organic Chemicals, Rochester, N. Y.
[3] The Squibb Institute for Medical Research, E. R. Squibb and Sons, New Brunswick, N. J.
[4] Fisher differential thermalyzer, model 260, employed through the courtesy of Dr. Omar Steward, Department of Chemistry, Duquesne University.