Synthesis and Biological Investigations of Some 5*H*-1,3,4-Oxadiazolo[3,2-*a*]pyrimidin-5-ones

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Received May 20, 1981, from the *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, A.R. Egypt.* Accepted for publication June 16, 1981.

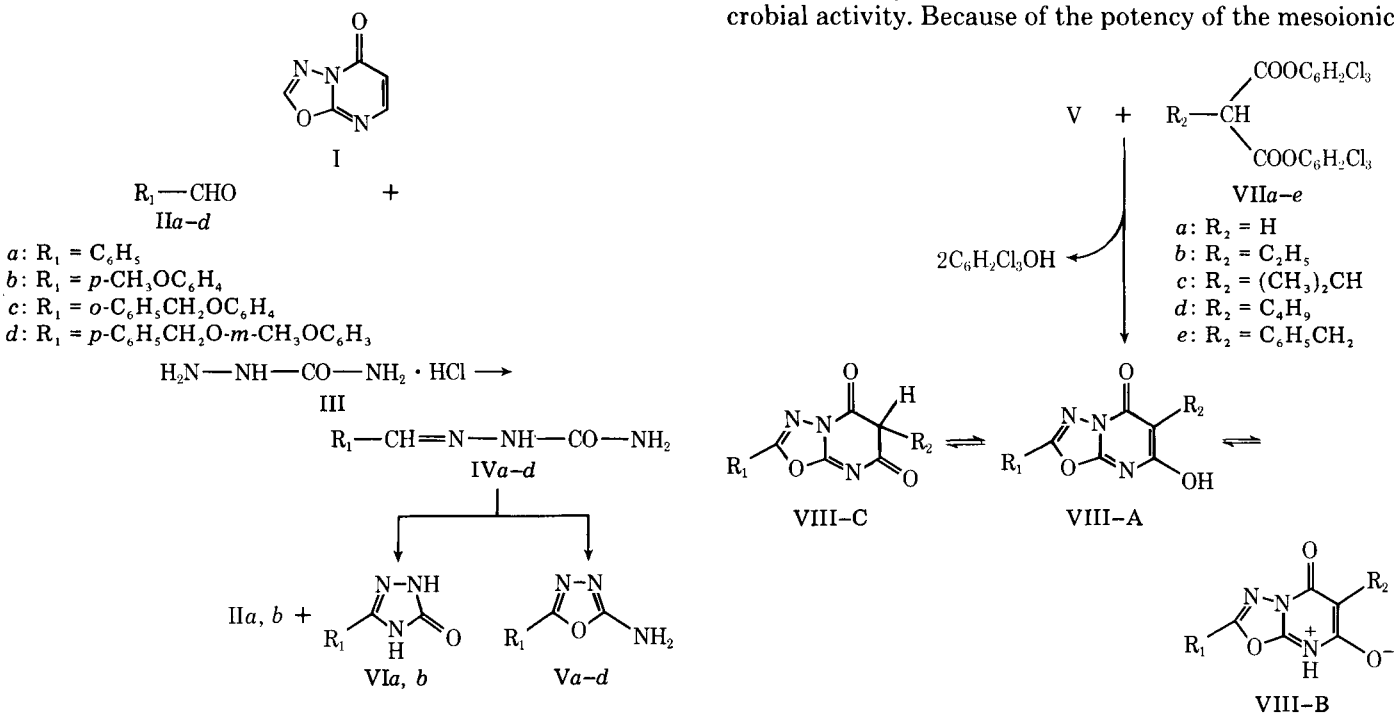
Abstract □ The synthesis of some substituted 7-hydroxy-5*H*-1,3,4-oxadiazolo [3,2-*a*]pyrimidin-5-ones, a class of bicyclics with unexplored pharmacotoxicological properties, is described. Reacting the 2-phenyl derivative with bis(2,4,6-trichlorophenyl)benzylmalonate afforded a linear pyrano-oxadiazolopyrimidinedione. The assigned structures were verified by IR, ^{1}H-NMR, and mass spectral studies. Six compounds of the series were screened for *in vitro* antibacterial and antifungal activities. The effect of four compounds on alkaline phosphatase enzyme was also examined.

Keyphrases □ 5*H*-1,3,4-Oxadiazolo[3,2-*a*]pyrimidin-5-ones—synthesis, screening for antibacterial and antifungal properties and phosphatase inhibition □ ^{1}H-NMR—characterization of 5*H*-1,3,4-oxadiazolo[3,2-*a*]pyrimidin-5-ones □ Mass spectrometry—characterization of 5*H*-1,3,4-oxadiazolo[3,2-*a*]pyrimidin-5-ones

A number of syntheses of substituted 5*H*-1,3,4-oxadiazolo[3,2-*a*]pyrimidin-5-ones (I) have been described in the literature. The 7-hydroxy derivative of I was first produced from 2-amino-1,3,4-oxadiazole and carbon suboxide (1). Ethyl 2-substituted-5-oxo-5*H*-1,3,4-oxadiazolo[3,2-*a*]pyrimidine-6-carboxylates were prepared by fusing 2-amino-5-substituted-1,3,4-oxadiazoles with ethyl ethoxymethylenemalonate and subsequent ring closure of the formed 2-(β,β-dicarbethoxyvinylamino)-1,3,4-oxadiazole derivatives (2). Analogously, 7-methyl-2-substituted-5*H*-1,3,4-oxadiazolo[3,2-*a*]pyrimidin-5-ones have been obtained from 2-aminooxadiazoles and ethyl acetoacetate (2). Reacting 2-amino-1,3,4-oxadiazoles with acetylenemono- or acetylenedicarboxylates afforded the 2-substituted or the 2-substituted-7-carboxylate derivatives of I, respectively (3). 6-Acetyl-2-phenyl-5*H*-1,3,4-oxadiazolo[3,2-*a*]pyrimidin-5-one was obtained *via* interaction of *N'*-2-(5-phenyl-1,3,4-oxadiazolyl)-*N*,*N*-dimethylformamidine with diketene (4). Furthermore, the preparation of some 6,7-dihydro derivatives of I was achieved either by cyclizing 3-(β-chloropropionyl)-2-imino-5-substituted-1,3,4-oxadiazolines (5) or through cycloaddition of diphenylketene with 2-arylideneamino-1,3,4-oxadiazoles (6). On the other hand, class II mesoionic 1,3,4-oxadiazolo[3,2-*a*]pyrimidine-5,6-diones, which can be regarded as isoelectronic and isosteric to xanthines, have been postulated among different bicyclic mesoionic heteroaromatic structures, called mesoionic purinone analogs (7).

There has been no report concerning the bioactivities of such compounds. In contrast, some bioisosteric sulfur analogs derived from 5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-one reportedly possess herbicidal and pesticidal potencies (8). In addition, a number of class-II mesoionic 1,3,4-thiadiazolo[3,2-*a*]pyrimidine-5,7-diones were either shown to display *in vitro* antibacterial activities (9, 10) or acted as inhibitors of adenosine-3',5'-monophosphate phosphodiesterase (11).

As a continuation of previous work (12), the synthesis of various 7-hydroxy-5*H*-1,3,4-oxadiazolo[3,2-*a*]pyrimidin-5-one derivatives was undertaken, and some representative compounds were screened for *in vitro* antimicrobial activity. Because of the potency of the mesoionic



Scheme I

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Table I—Substituted 7-Hydroxy-5*H*-1,3,4-Oxadiazolo[3,2-*a*]pyrimidin-5-ones (VIII)

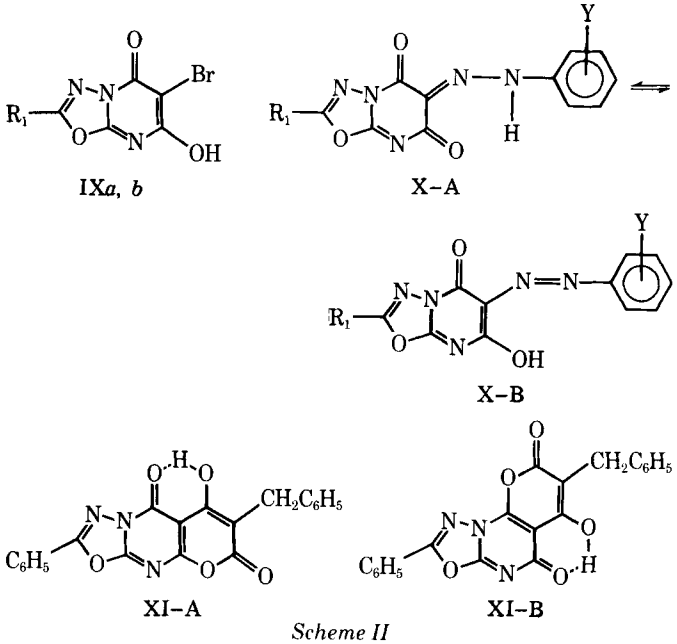
Compound	R_1	R_2	Melting Point (Recrystallization Solvent)	Yield, %	Molecular Formula	Analysis, % Calc.	Found	IR,[a] cm^{-1}
VIII*a*	C_6H_5	H	246–248° dec. (ethanol)	88	$C_{11}H_7N_3O_3$	C 57.6 H 3.1 N 18.3	57.3 3.0 18.4	3340–2500 (bm, OH and NH); a split band at 1715 (m, C=O), 1680 (s); 1615 (s); 1580 (s); 1545 (w); 1510 (m, C=N, C=C, and aromatics); 1255 (bs) shouldered at 1220; and 1015 (w, C–O–C)[b]
VIII*b*	C_6H_5	C_2H_5	265–268° dec. (ethanol)	70	$C_{13}H_{11}N_3O_3$	C 60.7 H 4.3 N 16.3	61.0 4.6 16.7	3300–2500 (b, OH and NH)[c]; 1670 (s, C=O) 1635 (w); 1605 (s), 1570 (m); 1540 (bs) shouldered at 1520; and at 1510 (C=N, C=C and aromatics); 1255 (s); and 1025 (m, C–O–C).
VIII*c*	C_6H_5	C_4H_9	208–210° (ethanol–water)	58	$C_{15}H_{15}N_3O_3$	C 63.15 H 5.3 N 14.7	63.3 5.6 14.3	3300–2500 (b, OH and NH)[c]; a split band at 1685 (s, C=O); 1655 (m); 1610 (m); 1560 (s) shouldered at 1575; 1540; and at 1525 (C=N, C=C, and aromatics); 1275 (m); and 1025 (m, C–O–C).
VIII*d*	*p*-$CH_3OC_6H_4$	H	246–248° dec. (ethanol)	85	$C_{12}H_9N_3O_4$	C 55.6 H 3.5 N 16.2	55.9 3.7 16.5	3350–2500 (bm, OH and NH); 1685 (m) shouldered at 1710 (C=O); 1620 (s); 1580 (m); 1550 (w); 1515 (s, C=N, C=C, and aromatics); 1265 (bs); and 1015 (m) (C–O–C)[b].
VIII*e*	*p*-$CH_3OC_6H_4$	C_2H_5	257–258° (ethanol)	83	$C_{14}H_{13}N_3O_4$	C 58.5 H 4.6 N 14.6	59.1 4.6 14.6	3300–2500 (bm) (OH and NH); 1680 (s, C=O), 1620 (s) shouldered at 1635; 1580 (w); 1540 (m), 1520 (s) (C=N, C=C, and aromatics), 1270 (s), and 1020 (m) (C–O–C)[b].
VIII*f*	*p*-$CH_3OC_6H_4$	$C_6H_5CH_2$	256–258° (ethanol)	86	$C_{19}H_{15}N_3O_4$	C 65.3 H 4.3 N 12.0	65.2 4.1 12.1	3350–2500 (bm, OH and NH); 1710 (s, C=O); 1660 (m); 1620 (s); 1575 (m); 1510 (m, C=N, C=C, and aromatics), 1260 (s), and 1020 (m) (C–O–C)[b].
VIII*g*	*o*-$C_6H_5CH_2OC_6H_4$	H	215–217° dec. (ethanol)	89.5	$C_{18}H_{13}N_3O_4$	C 64.5 H 3.9 N 12.5	64.2 4.0 12.0	3200–2400 (b, OH and NH)[d], a split band at 1685 (m, C=O); 1650 (s); 1605 (s), 1585 (w), 1560 (s, C=N, C=C and aromatics); 1265 (s); and 1025 (m, C–O–C).
VIII*h*	*o*-$C_6H_5CH_2OC_6H_4$	$(CH_3)_2CH$	198–199° (methanol–water)	68	$C_{21}H_{19}N_3O_4$	C 66.8 H 5.1 N 11.1	66.8 5.1 10.9	3240–2500 (b, OH and NH)[c]; a split band at 1685 (s, (C=O); 1650 (w); 1610 (m); 1555 (m) shouldered at 1540; 1520; 1495 (m, C=N, C=C and aromatics); 1260 (m); 1025 (m, C–O–C).
VIII*i*	*o*-$C_6H_5CH_2OC_6H_4$	$C_6H_5CH_2$	230–232° (ethanol)	94	$C_{25}H_{19}N_3O_4$	C 70.6 H 4.5 N 9.9	70.3 4.4 10.1	3200–2300 (b, OH and NH)[c]; 1670 (m, C=O); 1605 (s); 1575 (w); 1525 (s, C=N, C=C, and aromatics); 1260 (s); and 1035 (s, C–O–C).
VIII*j*	*p*-$C_6H_5CH_2O$-*m*-$CH_3OC_6H_3$	H	257–259° dec. (dimethylformamide–ethanol)	86	$C_{19}H_{15}N_3O_5$	C 62.5 H 4.1 N 11.5	62.5 4.5 11.1	3400–2500 (bm, OH and NH); a split band at 1720 (m) C=O); 1680 (s); 1620 (s); 1585 (m); 1525 (s) shouldered at 1550 and at 1510 (C=N, C=C and aromatics), 1250 (s) and 1020 (*m*, C–O–C)[b].

[a] The abbreviation b is broad, bm is broad medium, bs is broad strong, m is medium, and s is strong. [b] For potassium bromide disc. [c] Overlapped by the Nujol absorption bond.

thiadiazolopyrimidines, the similarity in structural features of the adopted ring system (I) to xanthines, and the reported association of antimicrobial activities with 1,3,4-oxadiazoles (13, 14) and pyrimidine derivatives (15–17), the effect on alkaline phosphatase enzyme was also studied. The susceptibility of the 1,3,4-oxadiazole moiety to attack with potassium hydroxide (2) and amines (18) may be important to the predicted bioactivity of the prepared compounds.

RESULTS AND DISCUSSION

Chemistry—The reactions outlined in Schemes I and II were followed for the synthesis of the desired compounds. The key intermediates, 2-amino-5-aryl-1,3,4-oxadiazoles (V) were prepared by first condensing the chosen aldehydes (II) with semicarbazide hydrochloride (III) in the presence of sodium acetate followed by oxidative ring closure of the resulting aldehyde semicarbazones (IV) with aqueous iodine–potassium iodide in presence of sodium carbonate (19). A previous report (20) showed that the exclusion of sodium carbonate in the cyclization of IV*a* and IV*b* leads to the formation of the corresponding 5-aryl-2,4-dihy-



IXa, b X-A X-B XI-A XI-B

Scheme II

dro-3*H*-1,2,4-triazol-3-ones (VI*a*) and (VI*b*) in low yields alongside their parent aldehydes II*a* and II*b*, respectively. The synthesis of the semicarbazones, IV*c* and IV*d*, and their respective oxadiazoles, V*c* and V*d*, have not been reported previously.

Reacting equimolar quantities of V with unsubstituted or monosubstituted bis(2,4,6-trichlorophenyl)malonates (VII) in boiling chlorobenzene afforded high yields of substituted 7-hydroxy-5*H*-1,3,4-oxadiazolo[3,2-*a*]pyrimidin-5-ones (VIII). The reaction involved an intermediate ketene–carboxylate which formed *in situ* from VII at elevated temperatures (21). The IR and ^{1}H-NMR data of VIII (Tables I and II) indicated mostly forms VIII-A and/or VIII-B rather than VIII-C. This is analogous to the structures previously assigned to some related hydroxypyridone systems (22).

The mass spectral data of representative compounds of VIII are recorded in Table II. Compounds VIII*a*, *d*, *e*, and *f* showed molecular ions of relatively low intensity (12–32%). Although the benzoyl cations constituted the base peak of the spectra of VIII*a*, *d*, and *e*, the spectrum of VIII*f* showed the *p*-methoxybenzoyl cation (*m/z* 135) at a lower abundance (56%). All mass spectra showed an ion corresponding to the loss of a fragment (*m/z* 43) from the parent molecule which might be HCNO.

The reactivity of position 6 in the 6-unsubstituted analogs of VIII towards electrophilic attack was considered in order to search for more diverse derivatives. Treatment of VIII*a* or VIII*d* with bromine yielded the corresponding 6-bromo derivatives IX*a* and IX*b*, respectively. Diazocoupling of VIII*a*, *d*, or *g* with either diazotized aniline or an appropriate diazotized sulfonamide in aqueous acetic acid medium provided 6-phenylazo- or 6-(4-substituted sulfonamidophenylazo) derivatives, respectively (X, Table III). The IR (KBr) spectrum of X*a* displayed a broad weak O–H and/or N–H stretching absorption band between 3150–2800 cm^{-1}, suggesting that the azo forms (X-A) exist in equilibrium with their hydrazono tautomers (X-B).

On the other hand, when VIII*a* was condensed with bis(2,4,6-trichlorophenyl)benzylmalonate (VII*e*) in refluxing bromobenzene, a single product was isolated for which the elemental analysis and molecular ion peak coincided with the tricyclic derivative XI-A or XI-B. The linear structure XI-A was shown to be consistent with the IR (KBr) spectrum, since it revealed the tertiary amide–carbonyl stretching absorption at 1690 cm^{-1}. If the angular isomer XI-B was formed it would have displayed this band at a lower frequency.

Determination of Antimicrobial Activity[1]—Compounds VIII*a*, *b*, *d*, *e*, *f*, and *g*[2] were tested for *in vitro* activity against four bacteria: *Staphylococcus aureus* (209 P), *Escherichia coli* (K802N), *Salmonella gallinarum* (595), and *Klebsiella pneumoniae* (SK&F 4200); and three fungi, *Candida albicans* (B-311, BC-759), and *Trichophyton mentagrophytes* (AAB-995). Microtiter twofold broth dilution methods were used to determine the minimum inhibitory concentrations of the compounds in micrograms per milliliter at which the growth of the test cultures are completely suppressed.

The antibacterial activity was determined in trypticase soy broth. The compounds were run at concentrations of 0.2–200 μg/ml. Log cultures of the bacterial strains were diluted so that the inoculum was 10^5 cfu/ml in the test. The microtiter plates were incubated at 37° overnight and observed for inhibition of growth.

The antifungal activity was determined in Sabouraud glucose broth and the compounds were run at concentrations of 0.2–200 μg/ml. Log cultures of the *Candida* strains were used for the inoculum which was 10^5 cfu/ml in the test. Spore suspensions in the glucose broth were prepared from cultures grown on glucose broth agar slants for the *T. mentagrophytes* inoculum. The plates were incubated at 30° and observed for growth at 24 and 48 hr.

Dimethyl sulfoxide–methanol mixture (1:50) was used as the solvent for the compounds. A control without the test compound was included for each organism. Gentamicin and amphotericin B were used as standard antibiotics for comparison against the bacterial and fungal species utilized, respectively.

None of the compounds exhibited antimicrobial activity.

Inhibition of Alkaline Phosphatase[3]—Compounds VIII*d*, *e*, *f*, and *g* were tested for *in vitro* inhibitory activity of alkaline phosphatase following a reported method (23).

Table II—PMR and Mass Spectrometric Data of Some Substituted 5*H*-1,3,4-Oxadiazolo[3,2-*a*]pyrimidin-5-ones (VIII)

Compound	^{1}H-NMR (δppm)	Mass Spectrum: *m/z* Values (Relative abundance, %)
VIII*a*	6.7 (s, 1H, H at C-6), and 7.9–8.75 (m, 5H, aromatic H)[a].	230 (5, M + 1), 229 (32, M$^+$), 201 (8), 187 (19, M-CH_2=C=O), 186 (85), 161 (24), 145 (37), 138 (14), 118 (21), 112 (11), 106 (14), 105 (100), 91 (23), 89 (19), 77 (27, $C_6H_5^+$).
VIII*d*	3.8 (s, 3H, CH_3O), 5.25 (s, 1H, H at C-6), 7.1 (d, 2H, *J* = 8 Hz, aromatic H), 7.9 (d, 2H, *J* = 8 Hz, aromatic H), and 11.0 (broad, OH).	260 (6, M + 1), 259 (30, M$^+$), 217 (20, M-CH_2=C=O), 216 (91), 201 (10), 186 (14), 175 (15), 158 (8), 149 (24, *p*-$CH_3OC_6H_4CNO^+$), 136 (14), 135 (100), 134 (8), 133 (45, *p*-$CH_3OC_6H_4$-CN^+), 105 (23).
VIII*e*	1.0 (t, 3H, *J* = 7Hz), CH_3-ethyl), 2.4 (q, 2H, *J* = 7 Hz, CH_2-ethyl), 3.85 (s, 3H, CH_3O), 7.1 (d, 2H, *J* = 8 Hz, aromatic H), 7.9 (d, 2H, *J* = 8 Hz, aromatic H), and 12.0 (broad, OH).	288 (5, M + 1), 287 (12, M$^+$), 245 (8), 244 (17), 229 (5), 223 (5), 216 (14), 201 (16), 191 (24), 186 (10), 149 (30, *p*-CH_3O-$C_6H_4CNO^+$), 136 (14) 135 (100), 134 (12), 133 (39, *p*-$CH_3OC_6H_5CN^+$), 105 (24), 77 (34), $C_6H_5^+$).
VIII*f*	3.75 (s, 2H, CH_2-benzyl), 3.85 (s, 3H, CH_3O), 7.2 (d, 7H, *J* = 8 Hz, aromatic H), 7.95 (d, 2H, *J* = 8 Hz, aromatic H), and 10.0 (broad, OH).	350 (8, M + 1), 349 (32, M$^+$, 306 (20), 278 (20), 277 (24), 264 (12), 247 (8), 218 (8, M-C_6H_5-$CH_2\dot{C}$=C=O), 215 (8), 149 (16, *p*-CH_3O-$C_6H_4CNO^+$), 139 (24), 135 (56), 133 (68, *p*-$CH_3OC_6H_5CN^+$), 103 (40), 91 (56, $C_6H_5CH_2^+$), 77 (40, $C_6H_5^+$), 44 (100).

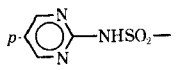
[a] Trifluoroacetic acid was used as solvent.

[1] Conducted by Smith Kline & French Laboratories, Philadelphia, Pa.

[2] Smith Kline & French Nos. 88347, 88350, 88349, 88351, 88355, and 88354, respectively.

[3] Conducted in the Department of Pesticides and Plant Protection, Faculty of Agriculture, University of Alexandria, A.R. Egypt.

Table III—2-Aryl-7-hydroxy-6-(*p*-substituted phenylazo)-5*H*-1,3,4-oxadiazolo[3,2-*a*]pyrimidin-5-ones (X)

Compound	R_1	Y	Melting Point (Recrystallization Solvent)	Yield, %	Molecular Formula	Analysis, % Calc.	Found	IR[b], cm^{-1}
X*a*[a]	C_6H_5	H	268–269° dec. (dimethylformamide–ethanol)	85	$C_{17}H_{11}N_5O_3$	C 61.3 H 3.3 N 21.0	61.0 3.3 21.3	3150–2800 (bw, OH and/or NH), 1735 (s, C=O), 1665 (m), a band split at 1610 (s), 1590 (s), 1575 (s), 1500 (s), 1460 (m, C=N, C=C and aromatics) a band split at 1225 (m) and at 1245 (w), and 1020 (m, C–O–C)[c].
X*b*	*p*-CH_3O-C_6H_4	*p*-H_2NSO_2-	288–290° dec. (dimethylformamide–water)	78.5	$C_{18}H_{14}N_6O_6S$	C 48.9 H 3.2 N 19.0 S 7.25	48.8 3.6 19.1 6.9	3370 (m), 3270 (m, NH and OH)[d], 1725 (s, C=O), 1650 (m), 1615 (s) shouldered at 1575, 1505 (s, C=N, C=C and aromatics), 1320 (m, asymmetric SO_2), 1255 (s, C–O–C), 1150 (s, symmetric SO_2), and 1025 (m, C–O–C).
X*c*	*p*-CH_3O-C_6H_4	*p*-  -$NHSO_2$-	304–305° dec. (dimethylformamide–ethanol)	34.5	$C_{22}H_{16}N_8O_6S$	C 50.8 H 3.1 S 6.2	50.6 3.5 6.0	3250–2400 (b, NH and OH)[d], 1720 (m, C=O), 1650 (w), a band split at 1605 (s) and 1575 (s, C=N and aromatics), 1250 (m, C–O–C), 1150 (s, SO_2), and 1030 (m, C–O–C) shouldered at 1010.
X*d*	*o*-C_6H_5-$CH_2OC_6H_4$	*p*-$H_2N-C(=NH)-NHSO_2$-	260° dec. (dimethylformamide–ethanol)	72.5	$C_{25}H_{20}N_8O_6S$	C 53.6 H 3.6 N 20.0 S 5.7	53.4 3.8 20.3 5.7	3500–3100 (m, with several splits, NH and OH)[d], 1715 (m, C=O), a band split at 1650 (m), 1620 (m), 1595 (s), 1580 (s), 1560 (s) and at 1520 (m, C=N, C=C, and aromatics), 1260 (s), and 1025 (s, C–O–C).

[a] ^{1}H-NMR (trifluoroacetic acid) δ: 7.3–8.25 (m, 10H, aromatic H) ppm. [b] b is broad, bm is broad-medium, m is medium, s is strong. [c] For potassium bromide disc. [d] Overlapped by the Nujol absorption band.

The butanol extractable alkaline phosphatase of the human placenta was partially purified as previously described for human liver alkaline phosphatase (24). The enzyme activity was measured at 25° by determining the hydrolysis rate of *p*-nitrophenyl phosphate using the change in absorbance at 410 nm with a spectrophotometer[4]. The reaction mixture contained 1.0 *M* tromethamine hydrochloride (pH 9.4), 10^{-3} *M* substrate, and 50 μl of enzyme in a final volume of 3 ml. The appropriate quantity of the compound dissolved in dimethyl sulfoxide (10^{-2}–10^{-4} *M* concentrations) was incubated with the enzyme in the reaction-buffer at room temperature for 10 min and the reaction was initiated by the addition of the substrate. A control containing dimethyl sulfoxide was included in all determinations.

No inhibition of enzyme activity was observed by any of the compounds tested.

EXPERIMENTAL[5]

Aldehyde Semicarbazones (IV)—These compounds were prepared by treating the appropriate aromatic aldehyde (II) with an equimolar amount of semicarbazide hydrochloride (III) and sodium acetate in aqueous ethanol. The semicarbazones were purified by recrystallization from ethanol.

***o*-Benzyloxybenzaldehyde Semicarbazone (IV*c*)**—The yield was 98% (mp 180–182°); IR: 3440, 3140 shouldered at 3340, 3260, and at 3200 (N–H), 1680 (C=O), 1580 (C=N, and aromatics), 1245, and 1020 (C–O–C) cm^{-1}.

Anal.—Calc. for $C_{15}H_{15}N_3O_2$: C, 66.9; H, 5.6; N, 15.6. Found: C, 66.6; H, 5.6; N, 15.9.

***p*-Benzyloxy-*m*-methoxybenzaldehyde Semicarbazone (IV*d*)**—The yield was 97% (mp 163–164°).

Anal.—Calc. for $C_{16}H_{17}N_3O_3$: C, 64.2; H, 5.7; N, 14.0. Found: C, 64.3; H, 5.8; N, 13.8.

2-Amino-5-aryl-1,3,4-oxadiazoles (V)—These were prepared from IV by a reported method (19).

2-Amino-5-(*o*-benzyloxyphenyl)-1,3,4-oxadiazole (V*c*)—This compound was recrystallized from ethanol, mp 207–209°. The yield was 59%; IR: 3250, 3080 (N–H), 1650 (C=N), 1590 (δN–H and aromatics), 1240, and 1025 (C–O–C) cm^{-1}.

Anal.—Calc. for $C_{15}H_{13}N_3O_2$: C, 67.4; H, 4.9; N, 15.7. Found: C, 67.5; H, 5.2; N, 16.0.

2-Amino-5-(*p*-benzyloxy-*m*-methoxyphenyl)-1,3,4-oxadiazole (V*d*)—This compound was recrystallized from aqueous ethanol, mp 209–210°. The yield was 34%; IR: 3360 (N–H), 1665 (C=N), 1580 shouldered at 1600 (δ N–H and aromatics), 1500, a band split at 1245 and at 1225, and 1030 (C–O–C) cm^{-1}.

Anal.—Calc. for $C_{16}H_{15}N_3O_3$: C, 64.6; H, 5.1; N, 14.1. Found: C, 64.5; H, 4.8; N, 13.6.

Substituted 7-hydroxy-5*H*-1,3,4-oxadiazolo[3,2-*a*]pyrimidin-5-ones (VIII, Table I)—The appropriate bis(2,4,6-trichlorophenyl)-malonate (VII, 1 mmole) was added in one portion to a hot suspension of the amine (V, 1 mmole) in chlorobenzene (15 ml). The mixture was refluxed for 30–60 min. After cooling the crystallized solid was filtered, washed with light petroleum (bp 40–60°), and dried. In some cases, the cooled reaction mixture was treated with an equal volume of light petroleum to precipitate all the product. The compound was recrystallized from the appropriate solvent.

6-Bromo-7-hydroxy-2-phenyl-5*H*-1,3,4-oxadiazolo[3,2-*a*]pyrimidin-5-one (IX*a*)—Bromine (0.05 ml, 1 mmole) was added in one portion to a stirred solution of VIII*a* (0.23 g, 1 mmole) in acetic acid (30 ml) at room temperature. The color of bromine was immediately discharged with liberation of hydrogen bromide and precipitation of the bromo derivative. After 2 hr at room temperature the product was filtered, washed with ethanol, dried, and recrystallized from acetic acid, mp 265–267° dec. The yield was 0.23 g (74.4%).

Anal.—Calc. for $C_{11}H_6BrN_3O_3$: C, 42.9; H, 2.0; Br, 25.9; N, 13.6. Found: C, 42.6; H, 2.2; Br, 26.0; N, 13.6.

6-Bromo-7-hydroxy-2-(*p*-methoxyphenyl)-5*H*-1,3,4-oxadiazolo[3,2-*a*]pyrimidin-5-one (IX*b*)—This compound was prepared by brominating VIII*d* (0.26 g, 1 mmole) with bromine (0.05 ml, 1 mmole) as described for IX*a*. It was recrystallized from acetic acid, mp 248–251° dec. The yield was 0.27 g (79.6%); IR (KBr): 3200–2300 (OH and NH), a band split at 1690 (C=O) and at 1660, a band split at 1615 and at 1600, 1510 shouldered at 1530 (C=N, C=C, and aromatics), 1270, and 1015 (C–O–C) cm^{-1}; mass spectrum: *m/z* (relative abundance, %), 339 (38) (M^+ of ^{81}Br), 338 (15), and 337 (35) (M^+ of ^{79}Br).

Anal.—Calc. for $C_{12}H_8BrN_3O_4$: C, 42.6; H, 2.4; Br, 23.6; N, 12.4. Found: C, 42.7; H, 2.6; Br, 24.0; N, 12.0.

7-Hydroxy-2-phenyl-6-phenylazo-5*H*-1,3,4-oxadiazolo[3,2-*a*]-pyrimidin-5-one (X*a*, Table III)—To a stirred solution of VIII*a* (0.23 g, 1 mmole) in ethanol (10 ml) at 10° was added a cold solution of aniline (0.09 ml, 1 mmole) and sodium nitrite (0.08 g, 1.1 mmole) in 50% aqueous acetic acid (10 ml). After stirring for 1 hr, the reaction mixture was re-

[4] Pye Unicam SP-100.

[5] Melting points were determined in open-glass capillaries and are uncorrected. IR spectra were recorded for Nujol mulls, unless otherwise specified, on a Beckman 4210; or for potassium bromide disks on a Perkin-Elmer 421 spectrophotometer. ^{1}H-NMR spectra were determined on a Varian EM 360 spectrometer using deuterated dimethyl sulfoxide as solvent, unless otherwise indicated, and tetramethylsilane as the internal standard. Mass spectra were obtained using a Varian Mat 111 (80 ev) instrument and peaks of relative intensity below 5% of the base peak were omitted. Microanalysis, for samples dried over phosphorus pentoxide at 70° under reduced pressure, was carried out at the Microanalytical Unit, University of Cairo, A.R. Egypt.

frigerated overnight and a yellow product was collected, washed with water, dried, and recrystallized.

2-Aryl-7-hydroxy-6-(*p*-substituted phenylazo)-5*H*-1,3,4-oxadiazolo [3,2-*a*]pyrimidin-5-ones (X*b–d*, Table III)—The appropriate sulfonamide (1 mmole) was dissolved in acetic acid (20 ml) and the solution was cooled to 5°. A cold solution of sodium nitrite (1.1 mmole) in water (5 ml) was added and the resulting diazonium salt solution was filtered into a stirred solution of the proper VIII (1 mmole) in acetic acid (50–100 ml) at 10°. The reaction mixture was then worked up as described for X*a*, and the yellow crude product crystallized from the suitable solvent.

Reaction of VIII*a* with Bis(2,4,6-trichlorophenyl)benzylmalonate. (VII*e*)—Equimolar amounts of VIII*a* (0.23 g, 1 mmole) and VII*e* (0.55 g, 1 mmole) were refluxed in bromobenzene (25 ml) for 3 hr. The product (XI-A) that separated out on cooling was filtered, washed with light petroleum, and dried. It was recrystallized from a large volume of boiling ethanol, mp 306–308°. The yield was 72%; IR (KBr): 3650–2900 (OH), 1750 (C=O, α-pyrone), 1690 (C=O, amide), a band split at 1620 and 1605, 1585, 1560, 1505 (C=N, C=C, and aromatics), 1255, and 1025 (C–O–C) cm^{-1}; mass spectrum: *m/z* (relative abundance, %) 387 (69) (M^+).

Anal.—Calc. for $C_{21}H_{13}N_3O_5$: C, 65.1; H, 3.4; N, 10.85. Found: C, 65.3; H, 3.7; N, 10.3.

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ACKNOWLEDGMENTS

The authors thank Dr. W. Stadlbauer, Institut für Organische Chemie der Karl-Franzens-Universität, Graz, Austria, for providing samples of active malonic esters and for spectral determinations. They also thank Dr. C. E. Berkoff, Director, Organic Chemistry, Smith Kline & French Laboratories, Philadelphia, Pa., for arranging screening of compounds, and Mr. A. Pavloff, Senior Medicinal Chemist, Research Chemistry, for providing the antimicrobial test report. Thanks are also extended to Professor N. Mansour and Mrs. M. M. Khattab, Department of Pesticides and Plant Protection, Faculty of Agriculture, University of Alexandria, A. R. Egypt, for the alkaline phosphatase screen test.

Influence of Benzalkonium Chloride on the Dissolution Rate Behavior of Several Solid-Phase Preparations of Cholesterol in Bile Acid Solutions

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Received April 28, 1980, from *The College of Pharmacy, The University of Michigan, Ann Arbor, MI 48109.* Accepted for publication June 2, 1981.

Abstract □ Cholesterol dissolution rate accelerators, such as benzalkonium chloride, function by reducing the interfacial barrier that exists between the negatively-charged bile acid micelle and the negatively-charged cholesterol surface. It has been proposed that this reaction is accomplished by the binding of the positively-charged accelerator to the negatively-charged micelle. An earlier report showed that different solid preparations of cholesterol give different dissolution rates under the same conditions and these differences can be primarily accounted for by variations in the interfacial transport constant (*P*). By using the rotating disk dissolution apparatus and the Levich theory it has been possible to study the dissolution behavior of different cholesterol solid phases as a function of the benzalkonium chloride concentration. It was shown that the ratios of *P* values for the different phases are relatively constant over the range of the accelerator concentrations. This suggests that the accelerators act primarily on the micelle to enhance dissolution rate.

Keyphrases □ Benzalkonium chloride—effect on dissolution of cholesterol preparations in bile acid solutions □ Dissolution—cholesterol preparations, effect of benzalkonium chloride, bile acid solutions □ Cholesterol—effect of benzalkonium chloride on dissolution, bile acid solutions □ Gallstone kinetics—effect of benzalkonium chloride on dissolution of cholesterol preparations in bile acid solutions

Physicochemical studies of gallstone (cholesterol) dissolution kinetics have been performed in these laboratories (1–7). Theoretical treatment (2) showed that *in vivo* dissolution of cholesterol gallstones occurred at much lower rates than expected if dissolution were totally diffusion controlled. It was proposed that interfacial factors may be

0022-3549/82/0200-0182$01.00/0