1-Aminoacyl-2,3-dihydro-4(1H)-quinazolinone Derivatives with Choleretic and Antifibrillatory Activity

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Twenty four new 1-aminoacyl-2,3-dihydro-4(1H)-quinazolinone derivatives were prepared and evaluated for their pharmacological properties. The compounds with a cyclic amino group showed a choleretic activity. Some substances displayed also antifibrillatory and antiphlogistic activity.

In the search for new compounds of possible pharmacological activity the 2,3-dihydro-4(1H)-quinazolinone (DHQ)[1] nucleus appeared to us very attractive, in that it contains the biologically important pyrimidine skeleton and may be also related to the 1,3-benzoxazine, many derivatives of which show pharmacological activities. The pharmacological and clinical importance of 4(3H)-quinazolinone derivatives is also well known.

At the beginning of our work very little was known about the chemistry[2,3] and pharmacology[4] of DHQ derivatives. While the work was in progress, some chemical and spectral properties[5-7] of DHQ derivatives were described and diuretic,[4,8] antihypertensive,[9] antihistaminic,[10] CNS stimulant[11] and depressant,[12] antipyretic, and hypotensive[12] properties of DHQ derivatives were reported.

The present paper deals with the synthesis and pharmacological evaluation of 1-aminoacyl-DHQ derivatives.[13]

Chemistry.—3-Aryl-, 2-methyl-3-phenyl-, 2-phenyl-3-methyl-, and 2,3-diphenyl-DHQ, synthesized by known methods[2,5] from anthranilamides and aldehydes, were employed as starting materials. Acylation with a little more than 1 equiv of chloroacyl chlorides in an inert solvent, such as dioxane or acetone, in the presence of an acid-binding agent, yielded the 1-chloroacyl-DHQ. Only 2,3-diphenyl-DHQ failed to give the expected product, but it reacted with chloroacetyl chloride to give 2-chloroacetamido-N-phenylbenzamide, as identified by comparison with an authentic sample (see Experimental Section).

Böhme and Böing[5] reported that by acetylation of 2,2-dimethyl-DHQ (I) with 2 equiv of acetyl chloride and 1 equiv of pyridine the hetero ring was cleaved and N-acetylanthranilic acid was obtained instead of 1-acetyl-2,2-dimethyl-DHQ, the facile ring cleavage of I being related to the cyclic aminal structure of the hetero ring. We decided therefore to test differently substituted DHQ in the reaction with acetyl chloride under different conditions, as summarized in Table I.

TABLE I

REACTION OF DHQ[a] DERIVATIVES WITH ACETYL CHLORIDE

DHQ	Exptl conditions	Isolated products
2,2-Dimethyl-[a] (I)	*b*	N-Acetylanthranilic acid[c]
	d	N-Acetylanthranilic acid[c] + I
	e, *f*	1-Acetyl-2,2-dimethyl-DHQ[a]
2,2,3-Trimethyl-[a] (II)	*b*	N-Acetylanthranilic acid[c]
	e	1-Acetyl-2,2,3-trimethyl-DHQ[h]
2,3-Dimethyl-[g] (III)	*b*	1-Acetyl-2,3-dimethyl-DHQ[h]
3-Methyl-[g] (IV)	*b*	1-Acetyl-3-methyl-DHQ[h]
3-Phenyl-[i] (V)	*d*, *j*, *k*	1-Acetyl-3-phenyl-DHQ[h]
2,2-Dimethyl-3-phenyl-[g] (VI)	*b*	2-Acetamido-N-phenylbenzamide[l]
	e	VI + unidentified products

[a] See ref 5. [b] Following Böhme and Böing,[5] *i.e.*, with 2 equiv of AcCl and 1 equiv of pyridine in $CHCl_3$, 24 hr at room temperature. [c] A. Kaufmann, *Ber.*, **42**, 3480 (1909). [d] With 1 equiv of AcCl and 1 mole of K_2CO_3 in dioxane, as in Experimental Section, General Method. [e] With 1 equiv of AcCl and 1 equiv of pyridine in $CHCl_3$, 24 hr at room temperature. [f] With 2 equiv of AcCl and 2 equiv of pyridine in $CHCl_3$, 24 hr at room temperature. [g] See Experimental Section. [h] See Table II. [i] See ref 2. [j] As in *b* substituting dioxane for $CHCl_3$. [k] As in *e* substituting dioxane for $CHCl_3$. [l] M. Körner, *J. Prakt. Chem.*, **36**, 155 (1887).

As can be seen, depending on the conditions, the acetyl-DHQ were obtained from III, IV, and V, whereas from I and II, either the acetyl-DHQ or N-acetylanthranilic acid was isolated; from VI the only product we could obtain was 2-acetamido-N-phenylbenzamide, a type of product we had already encountered when attempting to chloroacylate 2,3-diphenyl-DHQ. It appears therefore that in some instances the nature of the starting DHQ and, in others, the choice of the conditions are determinant factors for the course of the reaction.

1-Chloroacyl-DHQ (Table II) were allowed to react with secondary amines (morpholine, piperidine, diethyl- and dimethylamine) to yield the 1-aminoacyl derivatives (Table III). Their water-soluble hydrochlorides were used for the pharmacological screening.

Pharmacology (Table IV). Toxicity.—The acute toxicity was determined intraperitoneally in mice for all compounds. After injection of toxic doses of **3**, **5**, **10**, **13**, **15–17**, and **22** the animals showed symptoms of

(1) DHQ is for 2,3-dihydro-4(1H)-quinazolinone(s) throughout the paper.

(2) J. R. Feldman and E. C. Wagner, *J. Org. Chem.*, **7**, 31 (1942).

(3) T. A. Kilroe Smith and H. Stephen, *Tetrahedron*, **1**, 38 (1957).

(4) E. Cohen, B. Klarberg, and J. R. Vaughan, *J. Am. Chem. Soc.*, **81**, 5508 (1959).

(5) H. Böhme and H. Böing, *Arch. Pharm.*, **293**, 1011 (1960).

(6) M. G. Biressi, M. Carissimi, and F. Ravenna, *Tetrahedron Letters*, 3949 (1966).

(7) H. Böhme and H. Böing, *Arch. Pharm.*, **294**, 556 (1961).

(8) (a) American Cyanamid Co., U. S. Patent 3,201,398 (1965); *Chem. Abstr.*, **63**, 18114*c* (1965); (b) Parke, Davis and Co., U. S. Patent 3,186,992 (1965); *Chem. Abstr.*, **63**, 13282*b* (1965).

(9) Instituto De Angeli S.p.A., French Patent M 1893 (1963); *Chem. Abstr.*, **60**, 3956*h* (1964).

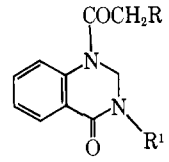
(10) C. H. Boehringer Sohn, French Patent M 2588 (1964); *Chem. Abstr.*, **61**, 16075*h* (1964).

(11) Shulton Inc., U. S. Patent 3,265,697 (1966); *Chem. Abstr.*, **65**, 15399*f* (1966).

(12) Rexall Drug Co., U. S. Patent 3,257,397 (1966); *Chem. Abstr.*, **65**, 8933*b* (1966).

(13) Since the writing of this manuscript, K. O. Kumura, T. Oine, Y. Yamada, G. Hayashi, and M. Nakama, *J. Med. Chem.*, **11**, 348 (1968), have reported the synthesis of some 1-acyl-DHQ with analgetic and anti-inflammatory activity.

TABLE II: 1-ACYL-2,3-DIHYDRO-4(1H)-QUINAZOLINONES



No.	R	R¹	Mp, °C	Recrystn solvent[a]	Yield, %	Formula	Analyses
1	Cl	C_6H_5	179–181	A	73	$C_{16}H_{13}ClN_2O_2$	C, H, N, Cl
2	Cl	$C_6H_4CH_3$-4	150–153	A	93	$C_{17}H_{15}ClN_2O_2$	C, H, N, Cl
3	Cl	$C_6H_4OCH_3$-4	133–135	A	42	$C_{17}H_{15}ClN_2O_3$	N, Cl
4	Cl	C_6H_4Br-4	158–160	A	93	$C_{16}H_{12}BrClN_2O_2$	N, Cl, Br
5	Cl	C_6H_4Cl-3	114–116	A	45	$C_{16}H_{12}Cl_2N_2O_2$	N, Cl
6	Cl	$C_6H_4CH_3$-2	125–126	A	90	$C_{17}H_{15}ClN_2O_2$	N, Cl
7	Cl	C_6H_5[b]	174–176[e]	A	60	$C_{17}H_{15}ClN_2O_2$	N, Cl
8	Cl	CH_3[c]	178–180	A	67	$C_{17}H_{15}ClN_2O_2$	N, Cl
9	CH_2Cl	C_6H_5	112–113	A	70	$C_{17}H_{15}ClN_2O_2$	C, H, N, Cl
10	CH_2Cl	$C_6H_4CH_3$-4	133–135	A	62	$C_{18}H_{17}ClN_2O_2$	C, H, N, Cl
11	H	C_6H_5	116–118	A	55	$C_{16}H_{14}N_2O_2$	C, H, N
12	H	CH_3	84–87	B	40	$C_{11}H_{12}N_2O_2$	C, H, N
13	H	CH_3[b]	111.5–114.5	B	46	$C_{12}H_{14}N_2O_2$	C, H, N
14	H	CH_3[d]	128–130	B	34	$C_{13}H_{16}N_2O_2$	C, H, N

[a] A = 95% EtOH, B = cyclohexane. [b] 2-Me. [c] 2-Ph. [d] 2,2-Me_2. [e] Lit.[13] mp 174–176°.

CNS excitation sometimes with tonic (**8**) or clonic convulsion (**1**, **2**, **4**, **6**).

After injection of toxic doses of **7**, **9**, **11**, **12**, **14**, **18–21**, **23**, and **24**, the animals, after an initial excitement, showed depression and died.

Choleretic Activity.—Only compounds with a cyclic amino group showed a significant choleretic activity. Particularly interesting was 1-morpholinoacetyl-3-phenyl-2,3-dihydro-4(1H)-quinazolinone hydrochloride (**1**). Its choleretic activity was compared in the rat to that of sodium dehydrocholate (**25**), of sodium α-(1-hydroxycyclohexyl)butyrate (**26**), and of sodium α-(1-hydroxy-4-phenylcyclohexyl)butyrate (**27**).

Compound **1** was active by the duodenal route at 6.25 mg/kg and was three times more active than **26** and **27**, and five times more active than **25**. It showed a choleretic effect also in the amount of dry residue. The choleretic activity was confirmed in the guinea pig, the rabbit, and the dog.

Antifibrillatory Activity.—Compounds **2**, **3**, **6**, **10**, **12–16**, **18**, **25**, **31**, and **38** protected against the ventricular fibrillation provoked by $CaCl_2$ on rats. The most active compounds were also tested on the rabbit heart against the fibrillation provoked by electric stimulation. All compounds showed at least as much activity as the quinidine.

Other Activities.—No activity was observed when the compounds were screened for smooth muscle relaxing activity, for local anesthetic activity, for effects on blood pressure and on respiration, for coronary vasodilatation, for antitussive activity, and for analgetic activity. Some compounds (**4**, **6–8**) exhibited antiphlogistic activity against formalin edema.

Experimental Section[14]

3-Methyl-2,3-dihydro-4(1H)-quinazolinone.—To a solution of 1.5 g (0.010 mole) of 2-amino-N-methylbenzamide in 15 ml of 95% EtOH were added 0.1 ml of 30% aqueous NaOH and 1 ml of 40% aqueous formaldehyde. The mixture was heated under reflux for 15 min and concentrated to dryness *in vacuo*. To the oily residue, dilute NaOH was added and the mixture was kept at room temperature until solidification had taken place. The solid was filtered and air dried; yield 1.1 g, mp 112–114°. Recrystallization (C_6H_6) gave 0.9 g, mp 112–114°. *Anal.* ($C_9H_{10}N_2O$) C, H, N.

2,3-Dimethyl-2,3-dihydro-4(1H)-quinazolinone.—A mixture of 1.5 g (0.010 mole) of 2-amino-N-methylbenzamide, 1.8 g (0.015 mole) of acetal, 0.1 g of *p*-toluenesulfonic acid monohydrate, and 15 ml of 95% EtOH was heated under reflux for 3 hr and the EtOH was removed *in vacuo*. The oily residue was treated with dilute NaOH and allowed to solidify. The solid was collected and air dried to give 1.1 g, mp 125–130°. Successive recrystallizations (C_6H_6, EtOAc) gave 0.6 g, mp 136–138°. *Anal.* ($C_{10}H_{12}N_2O$) C, H, N.

2,2-Dimethyl-3-phenyl-2,3-dihydro-4(1H)-quinazolinone.—To a solution of 2.1 g (0.010 mole) of 2-amino-N-phenylbenzamide in 10 ml of acetone was added 0.1 g of *p*-toluenesulfonic acid monohydrate. Soon a solid separated. The suspension was heated under reflux for 3 hr and cooled. The solid was filtered and air dried; yield 2.1 g, mp 254–256°. Recrystallization (Me_2CO) gave 1.4 g, mp 255–256°. *Anal.* ($C_{16}H_{16}N_2O$) C, H, N.

2-Methyl-3-phenyl-2,3-dihydro-4(1H)-quinazolinone.—A solution of 2.1 g (0.010 mole) of 2-amino-N-phenylbenzamide in 20 ml of warm 95% EtOH was acidified to pH 3.5 with ethanolic HCl and 2.36 g (0.020 mole) of acetal was added. The mixture was heated under reflux for 4 hr and diluted with H_2O (20 ml). The solid which separated on cooling was filtered and air dried; yield 1.8 g, mp 164–168°. Recrystallization from *i*-PrOH gave 1.5 g, mp 167–169° (lit.[13] mp 167–169°). *Anal.* ($C_{15}H_{14}N_2O$) C, H, N.

2,3-Diphenyl-2,3-dihydro-4(1H)-quinazolinone.—A mixture of 2.1 g (0.010 mole) of 2-amino-N-phenylbenzamide, 1.3 g (0.012 mole) of benzaldehyde, 0.1 ml of 30% aqueous NaOH, and 20 ml of 95% EtOH was heated under reflux for 1 hr and cooled. The solid was collected and recrystallized (EtOH) to give 2.4 g, mp 204–206°. *Anal.* ($C_{20}H_{16}N_2O$) C, H, N.

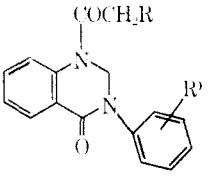
2-Chloroacetamido-N-phenylbenzamide.—To a stirred solution of 2.1 g (0.010 mole) of 2-amino-N-phenylbenzamide and 0.9 g (0.011 mole) of anhydrous NaOAc in 25 ml of AcOH at 40–50° was added 0.86 g (0.011 mole) of chloroacetyl chloride. The reaction mixture was stirred for 30 min after addition, cooled, and diluted with 25 ml of ice–water. The separated solid was collected and recrystallized (AcOH) to give 2.4 g, mp 189–191°. *Anal.* ($C_{15}H_{13}ClN_2O_2$) N, Cl.

1-Chloroacyl-2,3-dihydro-4(1H)-quinazolinones. General Method.—To a stirred mixture of DHQ (1 mole) and anhydrous K_2CO_3 (1.1 moles) in dioxane or acetone at steam bath temperature was added dropwise chloroacyl chloride (1.1 moles). The

(14) Melting points are uncorrected and were determined on a Kofler micro hot stage for all compounds except the 1-aminoacyl-DHQ hydrochlorides, for which sealed capillary tubes were used. When analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

TABLE III

1-AMINOACYL-2,3-DIHYDRO-4(1H)-QUINAZOLINONES



No.	R	R¹	Recrystn solvent[a]	Mp, °C	Yield, %	Formula	Analyses
1	Morpholino	H	A	135–137[b]	60	$C_{20}H_{21}N_3O_3$	C, H, N
			B	210–214		$C_{20}H_{21}N_3O_3 \cdot HCl$	C, H, N, Cl
2	Morpholino	4-CH_3	C	126–128	54	$C_{21}H_{23}N_3O_3$	C, H, N
			B	202–203		$C_{21}H_{23}N_3O_3 \cdot HCl \cdot H_2O$	N, Cl, H_2O[c]
3	Morpholino	4-OCH_3	D	144–145	47	$C_{21}H_{23}N_3O_4$	C, H, N, OMe
			E	212.5–213.5		$C_{21}H_{23}N_3O_4 \cdot HCl$	N, Cl
4	Morpholino	4-Br	F	181–182	70	$C_{20}H_{20}BrN_3O_3$	C, H, N, Br
			B	220 dec		$C_{20}H_{20}BrN_3O_3 \cdot HCl$	N, Cl
5	Morpholino	3-Cl	A	169–171	40	$C_{20}H_{20}ClN_3O_3$	C, H, N, Cl
			E	176–177		$C_{20}H_{20}ClN_3O_3 \cdot HCl$	N, Cl
6	Morpholino	2-CH_3	A	138–140	50	$C_{21}H_{23}N_3O_3$	C, H, N
			G	222–225 dec		$C_{21}H_{23}N_3O_3 \cdot HCl$	N, Cl
7	Morpholino	H[d]	C	133–135	68	$C_{21}H_{23}N_3O_3$	C, H, N
			G	127–130 dec		$C_{21}H_{23}N_3O_3 \cdot HCl$	N, Cl
8	Morpholino	[e]	F	159–161	40	$C_{21}H_{23}N_3O_3$	C, H, N
			H	243–245 dec		$C_{21}H_{23}N_3O_3 \cdot HCl$	N, Cl
9	Morpholinomethyl	H	C	113–114	46	$C_{21}H_{23}N_3O_3$	C, H, N
			B	203–206		$C_{21}H_{23}N_3O_3 \cdot HCl \cdot C_2H_5OH$	C, H, N, OEt
10	Morpholinomethyl	4-CH_3	I	133–135	42	$C_{22}H_{25}N_3O_3$	C, H, N
			B	213–215		$C_{22}H_{25}N_3O_3 \cdot HCl$	N, Cl
11	Piperidino	H	C	101–103	40	$C_{21}H_{23}N_3O_2$	C, H, N
			B	230–231		$C_{21}H_{23}N_3O_2 \cdot HCl$	N, Cl
12	Piperidino	4-CH_3	J	108–111	70	$C_{22}H_{25}N_3O_2$	C, H, N
			F	230 dec		$C_{22}H_{25}N_3O_2 \cdot HCl$	C, H, N, Cl
13	Piperidino	4-OCH_3	C	119–121	76	$C_{22}H_{25}N_3O_3$	C, H, N, OMe
			A	228–230		$C_{22}H_{25}N_3O_3 \cdot HCl$	N, Cl
14	Piperidino	3-Cl	J	139–141	80	$C_{21}H_{22}ClN_3O_2$	C, H, N, Cl
			F	215–217		$C_{21}H_{22}ClN_3O_2 \cdot HCl$	N, Cl
15	Piperidinomethyl	H	I	116–118	33	$C_{22}H_{25}N_3O_2$	C, H, N
			B	125 dec		$C_{22}H_{25}N_3O_2 \cdot HCl$	N, Cl
16	Piperidinomethyl	4-CH_3	C	113–115	65	$C_{23}H_{27}N_3O_2$	C, H, N
			E	204–206		$C_{23}H_{27}N_3O_2 \cdot HCl$	N, Cl
17	$(CH_3)_2N$	H	C	97–99	46	$C_{18}H_{19}N_3O_2$	C, H, N
			F	228–229		$C_{18}H_{19}N_3O_2 \cdot HCl$	N, Cl
18	$(CH_3)_2N$	4-CH_3	C	111–113	55	$C_{19}H_{21}N_3O_2$	C, H, N
			B	223–224		$C_{19}H_{21}N_3O_2 \cdot HCl$	C, H, N, Cl
19	$(CH_3)_2N$	4-OCH_3	D	116–117	75	$C_{19}H_{21}N_3O_3$	C, H, N, OMe
			F	112–113		$C_{19}H_{21}N_3O_3 \cdot HCl$	N, Cl
20	$(CH_3)_2N$	3-Cl	D	139–141	58	$C_{18}H_{18}ClN_3O_2$	C, H, N, Cl
			F	199–203		$C_{18}H_{18}ClN_3O_2 \cdot HCl$	N, Cl
21	$(C_2H_5)_2N$	H	K	77–80	45	$C_{20}H_{23}N_3O_2$	C, H, N
			F	138–140		$C_{20}H_{23}N_3O_2 \cdot HCl$	N, Cl
22	$(C_2H_5)_2N$	4-CH_3	K	76–79	73	$C_{21}H_{25}N_3O_2$	C, H, N
			F	211.5–212.5[f]		$C_{21}H_{25}N_3O_2 \cdot HCl$	N, Cl
23	$(C_2H_5)_2N$	4-OCH_3	L	124–125	68	$C_{21}H_{25}N_3O_3$	C, H, N, OMe
			F	144–147		$C_{21}H_{25}N_3O_3 \cdot HCl \cdot H_2O$	C, H, N, Cl, H_2O[c]
24	$(C_2H_5)_2N$	3-Cl	A	106–108	51	$C_{20}H_{22}ClN_3O_2$	C, H, N, Cl
			B	150 dec		$C_{20}H_{22}ClN_3O_2 \cdot HCl$	C, H, N, Cl

[a] A = *i*-PrOH, B = EtOH–Et_2O, C = cyclohexane, D = C_6H_6–cyclohexane, E = precipitated with ethanolic HCl from an ethereal solution of the base, F = Me_2CO, G = precipitated with ethanolic HCl from a Me_2CO solution of the base, H = distilled H_2O, I = C_6H_6–petroleum ether (bp 60–70°), J = EtOAc, K = ligroin, L = hexane. [b] From EtOAc or C_6H_6–petroleum ether, mp 128–130°. [c] Karl Fischer. [d] 2-Me. [e] 3-Me-2-Ph. [f] Monohydrate, mp 160–165°, from 10% aqueous Me_2CO. *Anal.* ($C_{21}H_{25}N_3O_2 \cdot HCl \cdot H_2O$) C, H, N, Cl, H_2O.[c]

mixture was stirred for 30 min after addition, cooled, and poured into H_2O. The solid which separated was collected and recrystallized (EtOH).

1-Aminoacyl-2,3-dihydro-4(1H)-quinazolinones. General Method.—A mixture of 1-chloroacyl-DHQ (1 mole) and secondary amine (2.2 moles) in C_6H_6 was heated under reflux for 3–5 hr or, when Me_2NH was employed, left at room temperature for 1–3 days. The reaction mixture was filtered to remove the secondary amine hydrochloride and the solution was extracted with dilute HCl. The acid extract was made alkaline with Na_2CO_3 solution and the separated base was collected and recrystallized. The hydrochloride salts were obtained either by adding

TABLE IV

PHARMACOLOGICAL ACTIVITIES OF 1-AMINOACYL-2,3-DIHYDRO-4(1H)-QUINAZOLINONE HYDROCHLORIDES

No.	Choleretic act., mg/kg[a,d]	Antifibrillatory act. mg/kg[b,d]	Antifibrillatory act. mg/l.[c,d]	LD_{50}, mg/kg ip	Other pharmacol act.
1	6.25	(48)	(10)	560[h]	
2	25	31	(10)	500[h]	
3	(130)	41		1300	*k*
4	45	(56)		450[h]	*l, m*
5	75	(190)		1500	*k*
6	30	(36)		300[h]	*l, m*
7	20	(25)		200[i]	*m*
8	35	44		350[j]	*m*
9	12.5	(31)		250[i]	
10	30	37		300	
11	18	(22)		180[i]	
12	15	10	0.81[f]	150[i]	
13	25	31		250	
14	(25)	12	1.8[f]	250[i]	
15	(8)	10	2.6[g]	80	
16	(15)	(5)[e]		150	
17	(25)	(31)		250	
18	(28)	10	6.2[g]	280[i]	
19	(20)	25		200[i]	
20	(30)	38		300[i]	
21	(25)	31		250[i]	
22	25	16	3.3[f]	250	
23	(20)	12		200[i]	
24	(20)	6	2.5[f]	200[i]	

[a] Dose which increased the bile flow to 50%. Maximum tested doses were $0.1LD_{50}$. Sodium dehydrocholate was active at 50 mg/kg. [b] Dose which prevented the cardiac arrhythmia in 50% of animals. Maximum tested doses were $0.12LD_{50}$. Procainamide was active at 50 mg/kg. [c] Concentration which reduced to 50% the heart sensitivity to the electric stimulation. Maximum tested doses were 10 mg/l. [d] Numbers in parentheses are maximum tested nonactive doses. [e] Higher doses were toxic. [f] Quinidine was active at 2.8 mg/l. [g] Quinidine was active at 6.1 mg/l. [h] Clonic convulsions. [i] Hypnosis. [j] Tonic convulsions. [k] Anticonvulsant activity. [l] Transient increase of arterial blood pressure and stimulant effect on respiration. [m] Inhibition of formalin edema of the paw.

the calculated amount of ethanolic HCl to a solution of the base in ether, benzene, acetone, or EtOH, or by dissolving the base in aqueous HCl and concentrating the solution until crystallization set in. Recrystallization from a suitable solvent (see Table III) may follow.

Pharmacological Methods. Animals.—NMRI albino mice (18–20 g) and Wistar albino rats (200–250 g) were used. For choleretic activity, 100-day-old Wistar albino female rats, 220–240 g, were used.

Acute Toxicity.—LD_{50} values were determined in mice intraperitoneally, and the mortality over 5 days was recorded. The animals were also observed for behavior and objective symptoms according to the Irwin[15] scheme.

Choleretic Activity.—Female rats, fasted for 14 hr and anesthetized with urethan, were used. The substances were injected into the duodenum. The bile flow was recorded 1 hr before and 1 hr after the administration of the compounds, by means of a graduated pipet connected to the cannulated choledochus.

Antifibrillatory Activity.—The compounds were given intravenously to rats anesthetized with pentobarbital sodium, and their ability to prevent cardiac arrhythmias induced by $CaCl_2$ was determined. Active compounds were then tested on rabbit heart by the method of Visentini.[16] The heart was stimulated with a frequency of 50/sec for 1 msec. The intensity which provoked the fibrillation was recorded before and after 20 min of perfusion with the testing compounds.

Other Tests.—All compounds were screened also for their antispasmodic activity "*in vitro*" following the methods described by Setnikar and Tirone,[17] and for their local anesthetic activity on the mouse tail according to Bianchi's method.[18] The analgetic activity was assayed in mice after oral administration, according to Bianchi and Franceschini.[19] Coronary vasodilatator activity on the isolated rabbit heart following the method of Setnikar, *et al.*,[20] was also determined.

Antimicrobial and antifungal activity, effects on blood pressure and on respiration, anticonvulsant activity, antitussive activity, and antiinflammatory activity were determined according to the methods previously described.[21]

(15) This scheme was discussed informally by S. Irwin at a Gordon Research Conference, New London, N. H., 1959.

(16) P. Visentini, *Arch. Ital. Sci. Farmacol.*, **4**, 16 (1954).

(17) I. Setnikar and P. Tirone, *Arzneimittel-Forsch.*, **16**, 1146 (1966).

(18) C. Bianchi, *Brit. J. Pharmacol.*, **11**, 104 (1956).

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(20) I. Setnikar, W. Murmann, and M. T. Ravasi, *Arch. Intern. Pharmacodyn.*, **131**, 187 (1961).

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Synthesis and Antiinflammatory Activity of 4-(*p*-Biphenylyl)-3-hydroxybutyric Acid and Related Compounds

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4-(*p*-Biphenylyl)-3-hydroxybutyric acid and about 50 related compounds are reported. The title compound showed pronounced antiinflammatory activity.

Some years ago as part of a program for the investigation of compounds related to mephenesin (I, R = *o*-tolyloxy; R′ = OH) and chlorphenesin (I, R = *p*-chlorophenoxy; R′ = OH), the formally related 4-aryloxy-3-hydroxybutyric acids (I, R = *o*-tolyloxy or *p*-chlorophenoxy; R′ = CO_2H) were prepared for routine biological screening.

$$RCH_2CHOHCH_2R'$$
I

Subsequently the series was extended and the unexpected observation was made that 4-(*p*-biphenylyloxy)-3-hydroxybutyric acid showed significant antiinflammatory activity in the uv erythema and rat paw tests. A systematic study of this group of compounds was therefore made (see Table I), but a product worthy of clinical study did not emerge.

The acids described in Table I were prepared starting from the aryloxychlorohydrins[1] (I, R = aryloxy; R′ = Cl) which were converted into the nitriles (I, R =

(1) O. Stephenson, *J. Chem. Soc.*, 1571 (1954).