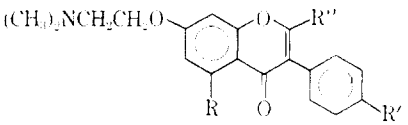
TABLE IV

DIALKYLAMINOALKOXYISOFLAVONES



Compd	R	R'	R''	Mp, °C[a]	λ_{max},[b] mμ	ε	Activity[c] A[d]	Activity[c] B[d]
XIX[e]	H	OCH_3	H	144–147	298	11,500	−/10	0/25
					259	28,800		
					249	28,200		
XX	H	OCH_3	CH_3	125–127	294	13,300	+/10	0/25
					248	29,200	−/5	
					242	28,900		
XXI	H	OCH_3	C_2H_5	97–100	294	14,000	±/10	0/25
					248	29,300		
					241	29,800		
XXII	H	OCH_3	C_6H_5	156–158	308	16,500	−/10	0/25
					236	30,000		
XXIII	H	H	H	162–164.5	297	12,000	−/10	0/25
					248	29,500		
XXIV	H	F	H	165–167	298	11,200	−/10	0/25
					248	27,300		
XXV	H	Cl	H	163–164			−/10	
XXVI	OH	OCH_3	C_2H_5	95–97	319	5,500	−/10	0/25
					285[f]			
					258	35,000		
XXVII	CH_3	OCH_3	C_2H_5	114–115	283	13,000	−/10	0/25
					277	13,000		
					250	30,500		
					243	30,500		

Compd	Crystn solvent	Formula	Calcd, %: C	H	N	N	Found, %: C	H	N	N
XIX	Acetone	$C_{20}H_{21}NO_4$	70.78	6.24	4.13		70.78	6.27	4.27	
XX	Acetone	$C_{21}H_{23}NO_4$	71.37	6.56	3.97		71.46	6.53	3.87	
XXI	Ether	$C_{22}H_{25}NO_4$	71.90	6.86	3.82		71.92	6.63	3.64	
XXII	Ether	$C_{26}H_{25}NO_4$	75.15	6.06	3.37		74.96	5.98	3.20	
XXIII	Acetone	$C_{19}H_{19}NO_3$	73.76	6.20	4.53		73.61	6.21	4.42	
XXIV	Acetone	$C_{19}H_{18}FNO_3$	69.70	5.54	4.28	5.80	69.78	5.59	4.32	5.90
XXV	Acetone	$C_{19}H_{18}ClNO_3$	66.37	5.27	4.07	10.32	66.69	5.45	4.08	10.50
XXVI	Ether	$C_{22}H_{25}NO_5$	68.91	6.57	3.65		68.78	6.58	3.72	
XXVII	Ether	$C_{23}H_{27}NO_4$	72.41	7.13	3.67		72.29	7.08	3.73	

[a–d] See corresponding footnote in Table I. [e] This compound is described by Siphar, S. A., Belgian Patent 633541 (1955) (Derwent No. 20622) and is said to have antilipemic properties. [f] Inflection.

Cholesterol-Solubilizing Agents Related to the Gallstone Problem

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Homologous series of three different types of higher alkyl substituted carboxylic acids were prepared and evaluated *in vitro* as cholesterol-solubilizing agents. The solubilization capability of the test compounds was found to increase with chain length, but this was accompanied by a decrease in solubility of the compound *per se*. Compounds of C_{14-16} side-chain length showed the greatest solubilization capability. Following oral administration, several quaternary nicotinic acids (Table I) which showed good *in vitro* cholesterol-solubilizing properties were excreted by way of the bile in the rat but not in the dog.

Pathologic cholesterol gallstones are known only in the human species except those produced by severe regulation of the diet of experimental animals.[1] The restriction of this affliction to man is accompanied by the observation that other species produce bile far below cholesterol saturation.[2] Indeed, isolated human gallstones are readily dissolved when placed either in the gallbladder of an animal or bathed in the bile of animals[3] but are generally quite resistant to dissolution in nonlithogenic human bile.[4] The cholesterol

(1) H. Dam and F. Christensen, *Acta Pathol. Microbiol. Scand.*, **30**, 236 (1952).

(2) C. G. Johnston and F. Nakayama, *Arch. Surg.*, **75**, 436 (1957).

(3) F. Nakayama and C. G. Johnston, *Proc. Soc. Exptl. Biol. Med.*, **104**, 73 (1960), and references therein.

(4) A few reports of spontaneous disappearance of gallstones in humans, presumably by dissolution, have been recorded: J. F. Linsman and E. Corday, *J. Am. Med. Assoc.*, **171**, 1098 (1959).

dispersed in human bile is therefore precariously close to crystallization which could occur if any of a number of conditions were unfavorably altered.

Aside from metabolically reducing the amount of cholesterol appearing in the bile, the gallstone problem could most reasonably be approached by attempting to render human bile more capable of holding cholesterol in solution. This might be accomplished by (a) altering biliary pH, ionic environment, protective proteins, and the like to minimize precipitation and concretion; (b) preferentially increasing the concentration of the bile salts, fatty acids, and phospholipids in the bile; or (c) administering an oral agent capable of solubilizing cholesterol in bile to a higher degree than normal. This paper describes our preliminary efforts to find an agent of the type described under (c).

The investigation was planned to proceed through three successive stages. First, *in vitro* screening techniques would be used to find compounds capable of cholesterol solubilization. Secondly, compounds would be evaluated *in vivo* to determine biliary excretion following oral administration. Those compounds detected in substantial quantities in the bile of dogs would proceed to the third-stage evaluation. This would be concerned with the relationship of the excreted form of the agent and the cholesterol-carrying capacity of the bile of higher mammals.

Methods

In Vitro **Studies. Test A.**—The light-scattering properties of serial dilutions of cholesterol suspensions in solutions containing test compound was measured according to Davis.[5] The amount of cholesterol solubilized was obtained from the plot of light-scattering *vs.* cholesterol concentration.

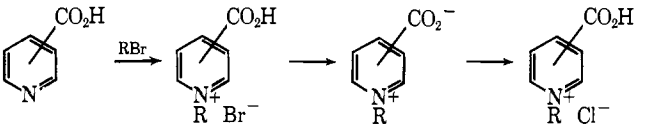
Test B.—Gallstones obtained from a single gallbladder and weighing approximately 200 mg each were soaked in water. Each day the gallstone was removed from the water, blotted dry, and weighed in a small covered dish. The procedure was repeated until the weight varied no more than 0.2 mg. Stones prepared in this manner were added to 5 ml of test solution formed from 0.25% of test compound in 0.1 *M* phosphate buffer at pH 7.8. The tubes were stoppered and rocked at 25 reciprocations/min for 60 hr at 37°. The change in weight was recorded.

In Vivo **Studies.**—Bile-fistula rats weighing 450–500 mg were employed. A polyethylene cannula from the proximal portion of the common bile duct was led through a stab wound in the right dorsal side of the body wall. Animals were confined in a Bollman-type restraining cage. The bile was collected continuously in a graduated centrifuge tube. Food and water were given *ad libitum*. The compounds were administered in a single dose intragastrically, and bile samples were taken for 2 days. The apparent concentration of sample was determined by comparison of ultraviolet spectrophotometric data with that of the administered drug.[6]

Results and Discussion

Quaternary Nicotinic Acids.—The first compounds tested were a series of alkyl quaternary nicotinic acids which have the feature of a strong chromophore in the ultraviolet spectral region, greatly aiding in their detection in bile. A literature search revealed that, although many relatively long-chain quaternary salts of nicotinamide,[7] isonicotinamide,[8] ethyl nicotinate,[9] and isonicotinate[8] are described, the same is not true for quaternary derivatives of nicotinic and isonicotinic acids. Only three examples of such compounds with chain length greater than C_8 (two C_{16} and one C_{18})[8,10] were found in the literature.

The even-number-carbon chain, decyl to octadecyl quaternary derivatives of nicotinic acid and the dodecyl and tetradecyl derivatives of isonicotinic acid, were prepared in about 50% yield by heating together the acid and alkyl bromide under controlled conditions. In certain examples the bromide salt (infrared absorption at 3.6–4.1 and 5.8 μ) was converted to the inner salt (infrared absorption 6.1 μ) by means of a basic, ion-



exchange resin. Treatment of the inner salt with hydrogen chloride gave the chloride salt. Yields were very low when the chloride salt was prepared directly using alkyl chloride.

Sodium 3-pyridinesulfonate reacted with *n*-decyl and *n*-dodecyl bromides to form 1-alkyl-3-pyridinium sulfonates[11] (strong bands at 8.1, 9.6, and 9.7 μ).

The quaternary pyridinium salts that were prepared are listed in Table I. Cholesterol-solubilizing properties of representative members of the series are given in Table II. Most of these compounds were excreted in the bile of rats. The same compounds, tested in dogs at a comparable dosage level, were not excreted in the bile. Since the dog has a biliary system more analogous to the human than does the rat, the negative findings in dogs discouraged further investigation of this series of compounds.

Other Homologous Series.—Bile contrast media are usually iodinated phenyl compounds that utilize the radioopaque feature of iodine.[12] We have synthesized compounds related to such agents although devoid of the iodine atoms. The modifications brought together the increased cholesterol-solubilizing feature of a long side chain and the known absorption–excretion properties of bile visualization compounds.

As an example, Archer and co-workers[13] prepared *p*-aminobenzenesulfonamides of the type p-$NH_2C_6H_4SO_2NHCHRCO_2H$ in which R was C_{2-6}. We have prepared the higher normal (saturated) homologs C_8, C_{10}, C_{12}, and C_{14} and have noted improved cholesterol solubilization *in vitro* with increased chain length. Higher members were not formed under the reaction conditions that gave the compounds to C_{14}. The members of this series and their cholesterol-solubilizing capacities are given in Table III.

The phenoxyalkanoic acid series of the type p-$RC_6H_4OCR_1R_2CO_2H$ where R is alkyl or acyl and R_1 and R_2 are H or alkyl was prepared and studied for cholesterol solubilizing value. Data are given in Table IV.

(5) W. W. Davis, *Trans. N. Y. Acad. Sci.*, **18**, 123 (1955).

(6) The identity of the test compound and the product excreted was not established.

(7) M. F. Zienty, *J. Am. Pharm. Assoc., Sci. Ed.*, **37**, 99 (1948).

(8) W. Ciusa and A. Buccelli, *Gazz. Chim. Ital.*, **88**, 393 (1958); *Chem. Abstr.*, **53**, 20059 (1959).

(9) G. H. Harris, *et al.*, *J. Am. Chem. Soc.*, **73**, 3959 (1951).

(10) Alkylation of isonicotinic acid has been reported to proceed in 1% yield: X. Mamaliga, *Comun. Acad. Rep. Populare Romine*, **5**, 1583 (1955); *Chem. Abstr.*, **50**, 16461 (1956); G. R. Bauwin and F. X. Grossi, U. S. Patent, 2,979,863 (1961); *Chem. Abstr.*, **55**, 27746 (1961).

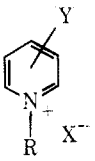
(11) H. Meyer, *Monatsh. Chem.*, **24**, 195 (1903), reported 1-methyl-3-pyridinium sulfonate.

(12) S. Archer, *Ann. N. Y. Acad. Sci.*, **78**, 720 (1959).

(13) S. Archer, J. O. Hoppe, T. R. Lewis, and M. N. Haskell, *J. Am. Pharm. Assoc., Sci. Ed.*, **40**, 143 (1951).

TABLE I

SUBSTITUTED PYRIDINIUM SALTS



No.	R	Y	X⁻	Mp, °C[a]	Yield, %	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	Procedure[b]
1	n-$C_{10}H_{21}$	3-CO_2H	Br	178–179	38	$C_{16}H_{26}BrNO_2$	55.81	7.61	4.06	55.11	7.60	3.92	A
2	n-$C_{12}H_{25}$	3-CO_2H	Br	176–178	27	$C_{18}H_{30}BrNO_2$	58.05	8.12	3.76	58.20	8.24	3.70	A
3	n-$C_{14}H_{29}$	3-CO_2H	Br	171–173	67	$C_{20}H_{34}BrNO_2$	59.99	8.55	3.49	60.20	8.31	3.85	B
4	n-$C_{16}H_{33}$	3-CO_2H	Br	175–176	30	$C_{22}H_{38}BrNO_2$	61.66	8.94	3.26	61.89	8.94	3.04	A
5	n-$C_{18}H_{37}$	3-CO_2H	Br	172–173	42	$C_{24}H_{42}BrNO_2$	63.14	9.28	3.06	63.70	8.72	3.07	A
6	n-$C_{12}H_{25}$	3-CO_2H	Cl	175–176	32	$C_{18}H_{30}ClNO_2$	65.93	9.22	4.27	66.23	9.39	4.11	A
7	n-$C_{14}H_{29}$	3-CO_2H	Cl	208–209	85	$C_{20}H_{34}ClNO_2$	67.48	9.62	3.94	67.62	9.38	3.76	C
8	$C_{12}H_{23}$[c]	3-CO_2H	Cl	197–198	70	$C_{18}H_{28}ClNO_2$	66.31	8.66	4.30	66.49	8.87	4.52	B
9	n-$C_{12}H_{25}$	3-CO_2H	I	145–147	21	$C_{18}H_{30}INO_2$	51.55	7.21	3.31	51.50	7.35	3.72	A
10	n-$C_{14}H_{29}$	3-CO_2^-		185 dec	85	$C_{20}H_{35}NO_2$			4.38			4.57	C
11	n-$C_{12}H_{25}$	4-CO_2H	Br	255–256	47	$C_{18}H_{30}BrNO_2$	58.05	8.12	3.76	58.02	7.85	3.83	B
12	n-$C_{14}H_{29}$	4-CO_2H	Br	250–252	52	$C_{20}H_{34}BrNO_2$	59.99	8.55	3.49	59.87	8.79	3.30	B
13	n-$C_{12}H_{25}$	4-CO_2^-		205 dec	81	$C_{18}H_{29}NO_2 \cdot H_2O$	69.86	10.22	4.53	69.89	10.26	4.73	C
14	n-$C_{10}H_{21}$	3-SO_3^-		197–198	27	$C_{15}H_{25}NO_3S$	60.16	8.41	4.67	60.33	8.37	5.02	D
15	n-$C_{12}H_{25}$	3-SO_3^-		200–201	61	$C_{17}H_{29}NO_3S$	62.35	8.92	4.28	62.38	8.82	4.44	D

[a] Melting points were taken with a Fisher-Johns apparatus and are uncorrected. [b] See experimental Section. [c] $C_{12}H_{23}$ is $CH_2CH{-}CHCH_2C(CH_3)_2CH_2C(CH_3)_3$.

TABLE II

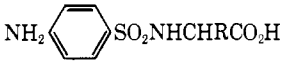
CHOLESTEROL SOLUBILIZATION BY PYRIDINIUM SALTS

No.[a]	Solubility[b]	Cholesterol dissolved *in vitro*[c]	Wt of compd given to rats[d]	No. of rats	No. of rats evaluated[e]	Average recovery of compd[e,f]
1	Very sol	0.35	100	3	3	22
3	10	1.05	100	4	2	36
4	5	1.05	100	4	4	42
5	Insol	...	60	3	2	16
9	20	0.60	200	3	1	39
11	5	0.65	100	4	4	36
15	Insol	...	100	4	[g]	...

[a] Refers to Table I. [b] Measured in milligrams per milliliter in phosphate buffer (pH 8.0) at 38°. [c] Measured in milligrams per milliliter (0.5% solution of test compound; see Methods, *in vitro* studies, test A. [d] Measured in milligrams; see Methods, *in vivo* studies. [e] After 48 hr with no interruption in continuous collection. [f] Measured in milligrams. [g] Compound toxic; all animals died.

TABLE III

CHOLESTEROL SOLUBILIZATION BY SULFONAMIDES



No.	R[a]	Cholesterol dissolved *in vitro*[b]	Gallstone dissolved *in vitro*[c]
1	n-C_8H_{17}	0.28	+0.1
2	n-$C_{10}H_{21}$	0.28	−2.9
3	n-$C_{12}H_{25}$	0.32	−21.0
4	n-$C_{14}H_{29}$	0.26	−19.7

[a] Characterized by comparison of spectrochemical properties with reported lower homologs. [b] Measured in milligrams per milliliter (0.33% solution of test compound; see Methods, *in vitro* studies, test A). [c] Measured in milligrams (0.25% solution of test compound; see Methods, *in vitro* studies, test B).

None of the compounds of Tables III or IV proved particularly effective as cholesterol solubilizers *in vitro*. Preliminary pharmacological excretion studies on representative compounds of Tables III and IV in rats did not warrant further investigation of these compounds.

Experimental Section

Substituted Pyridinium Salts (Table I). General Procedures. A.—A mixture of 1 mole of nicotinic acid and 1 mole of n-alkyl bromide was heated at 150° for 24 hr. After cooling to 100°, about 500 ml of ethanol was added and the insoluble nicotinic acid was removed by filtration. The alcohol solution was diluted with 500 ml of acetone and 1 l. of ether; the bromide salt was deposited on cooling.

B.—A mixture of 1 mole of isonicotinic acid and 1 mole of n-alkyl bromide was placed in a 3-l. flask equipped with a stirrer. The mixture was heated (190–220°) until an exothermic reaction ensured and then was maintained at 190° for 20–60 min. The product was isolated as in procedure A. The yield by procedure B was better than by procedure A.

C.—The product from procedure A or B dissolved in 400 ml of ethanol was passed through a column (3 in. × 4 ft) containing Amberlite IR 45 ion-exchange resin, hydroxide form.[14] The column was washed with 2 l. of ethanol, and the alcohol solution was concentrated to dryness under reduced pressure. The residue was digested with acetone, and the inner salt was collected by filtration.

The hydrochloride salt was prepared by dissolving the inner salt in 400 ml of ethanol and adding HCl at 10°. The product that separated was collected by filtration and recrystallized from ethanol.

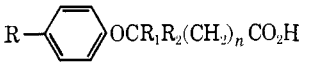
1-Alkylpyridinium 3-sulfonate (Procedure D).—A mixture of 0.2 mole of 3-pyridinesulfonic acid sodium salt and 0.2 mole of the n-alkyl bromide was heated at 150° for 24 hr or at 190° for 20 min. After cooling, the residue was mixed with 300 ml of ethanol, and the NaBr was removed by filtration. The alcohol solution was concentrated to near dryness, and the product was recrystallized from ethyl acetate.

2-Alkylsulfonamides (Table III) were prepared from the appropriate α-amino acid dissolved in 2 *N* NaOH and *p*-acetaminobenzenesulfonyl chloride followed by acid hydrolysis according

(14) The column was regenerated with 25% NaOH solution followed by water and absolute ethanol.

TABLE IV

4-SUBSTITUTED PHENOXYACETIC ACIDS



No.	R	R_1	R_2	n	Mp °C	Formula	Calcd, % C	Calcd, % H	Found, % C	Found, % H	Cholesterol dissolved *in vitro*[a]
1	$CH_3(CH_2)_4CO$	H	H	0	114–115	$C_{14}H_{18}O_4$	67.18	7.28	66.18	7.19	0
2	$CH_3(CH_2)_4CO$	H	CH_3	0	68–69	$C_{15}H_{20}O_4$	68.16	7.62	68.39	7.81	0
3	$CH_3(CH_2)_4CO$	CH_3	CH_3	0	93–94	$C_{16}H_{22}O_4$	69.04	7.96	69.24	8.18	0
4	$CH_3(CH_2)_4CO$	H	H	1	112–113	$C_{15}H_{20}O_4$	68.16	7.62	67.84	7.40	0
5	$CH_3(CH_2)_6CO$	H	H	0	121–122	$C_{16}H_{22}O_4$	69.04	7.96	68.75	7.80	0
6	$CH_3(CH_2)_6CO$	H	CH_3	0	68–69	$C_{17}H_{24}O_4$	69.83	8.27	69.50	8.20	0
7	$CH_3(CH_2)_6CO$	CH_3	CH_3	0	83–84	$C_{18}H_{26}O_4$	70.56	8.55	70.30	8.74	0
8	$CH_3(CH_2)_6CO$	H	H	1	109–110	$C_{17}H_{24}O_4$	69.83	8.27	69.70	8.14	*b*
9	$CH_3(CH_2)_5$	H	H	0	91–92	$C_{14}H_{20}O_3$	71.15	8.53	70.97	8.70	*b*
10	$CH_3(CH_2)_5$	H	CH_3	0	39–40	$C_{15}H_{22}O_3$	71.96	8.85	71.75	9.02	0.25
11	$CH_3(CH_2)_5$	CH_3	CH_3	0	38–39	$C_{16}H_{24}O_3$	72.69	9.15	72.59	9.16	0.35
12	$CH_3(CH_2)_7$	H	H	0	91–92	$C_{16}H_{24}O_3$	72.69	9.15	72.81	9.17	*b*
13	$CH_3(CH_2)_7$	H	CH_3	0	45–46	$C_{17}H_{26}O_3$	73.34	9.41	73.35	9.52	0.35[c]
14	$CH_3(CH_2)_7$	CH_3	CH_3	0	35–36	$C_{18}H_{28}O_3$	73.93	9.65	74.06	9.77	*b*

[a] Measured in milligrams per milliliter (0.5% solution of test compound; see Methods *in vitro* studies, test A). [b] Compound not soluble in phosphate buffer. [c] Tested as the sodium salt.

to the procedure of Archer.[13] The condensation proceeded with amino acids NH_2CHRCO_2H, where R equals C_8, C_{10}, C_{12}, and C_{14}. When higher side-chain amino acids were used under the reported reaction conditions, no reaction took place; when drastic conditions were employed, the corresponding diketopiperazines were formed.[15] The reported compounds and those of Table III showed comparable infrared and ultraviolet spectra.

Substituted Phenoxyacetic Acids (Table IV).—4-Acylphenols were prepared according to the procedure of Kindler, *et al.*[16] These compounds were reduced to the corresponding 4-alkylphenols according to the method of Sandulesco and Girard.[17]

The straight-chain and monomethylcarboxylic acids of Table IV were prepared in the following way. A solution of 0.1 mole of the appropriate phenol, 0.2 mole of KOH, and 200 ml of ethanol was heated to boiling. The appropriate bromocarboxylic acid (0.1 mole), dissolved in 50 ml of ethanol, was added dropwise, and the resulting solution was heated under reflux for 6 hr. The reaction mixture was cooled, acidified with dilute HCl, and concentrated under reduced pressure. The residue was extracted with ether, and, after being dried, the ether was removed to give a waxy residue. The product was recrystallized from petroleum ether (bp 60–70°) or $CHCl_3$–petroleum ether.

The dimethyl compounds **3**, **7**, **11**, and **14** of Table IV were prepared according to the procedure of Julia, *et al.*,[18] as follows. A solution of 0.1 mole of the appropriate phenol and 0.6 mole of KOH dissolved in 400 ml of acetone was cooled to 15°. About 20 ml of $CHCl_3$ was added dropwise, while the temperature was held below 30°. The mixture was heated under reflux for 1 hr and added to 1 l. of ice water. The solution was made acidic with 6 *N* HCl and extracted with ether. The ether solution was extracted with three 300-ml portions of 5% $NaHCO_3$. The basic solution was made acidic with 6 *N* HCl and again extracted with ether. The ether solution was treated with charcoal and anhydrous Na_2CO_3. After filtration and removal of the ether, the residue was recrystallized from petroleum ether (60–70°).

Acknowledgment.—The authors wish to thank Drs. S. M. Chernish and J. B. Hammond for supplying gallstones and human bile specimens and for their enthusiasm related to this project.

(15) This study was done by Ronald E. Hackler.

(16) K. Kindler, H. Oelschlager, and P. Henrich, *Arch. Pharm.*, **287**, 210 (1954).

(17) G. Sandulesco and A. Girard, *Bull. Soc. Chim. France*, **47**, 1300 (1930).

(18) M. Julia, M. Baillarge, and G. Tschernoff, *ibid.*, 776 (1956).