and 100 ml of water was added. An oil separated which was extracted into ether. Removal of the ether followed by distillation gave 23.9 g (80% yield) of 2-chloro-3-cyano-4-trifluoromethyl-6-methylpyridine, bp 85° (0.05 mm).

Anal. Calcd for $C_8H_4ClF_3N_2$: C, 43.55; H, 1.82; N, 12.69. Found: C, 43.19; H, 2.12; N, 12.24.

Dehalogenation by the same procedure used for the preparation of 3-cyano-6-methylpyridine gave 3-cyano-4-trifluoromethyl-6-methylpyridine in 52% yield, bp 50–52° (0.02 mm).

Anal. Calcd for $C_8H_5F_3N_2$: C, 51.61; H, 2.70; N, 15.04. Found: C, 51.54; H, 2.75; N, 15.06.

3-Cyano-4-methoxymethyl-6-methylpyridine.—Dehalogenation of 2-chloro-3-cyano-4-methoxymethyl-6-methylpyridine[43] by the same procedure used for the preparation of 3-cyano-6-methylpyridine gave the product in 66% yield, bp 150–155° (0.06–0.08 mm), mp 44–45°.

Anal. Calcd for $C_9H_{10}N_2O$: C, 66.65; H, 6.22; N, 17.27. Found: C, 66.29; H, 6.14; N, 17.30.

3-(5-Tetrazolyl)pyridine N-Oxide.—A solution containing 14.7 g (0.1 mole) of 5-(3-pyridyl)tetrazole, 75 ml of acetic acid, and 225 ml of 30% H_2O_2 was maintained at 90° for 24 hr. On cooling and triturating with ether there was obtained 11.1 g of crude product, mp 235° dec. Recrystallization from water gave 6.0 g (37% yield) of purified product, mp 248° dec.

5-(3-Pyridylmethyl)tetrazole Hydrochloride.—A mixture of 20 g of acetic acid, 26 g (0.22 mole) of 3-pyridylacetonitrile,[45] 100 ml of *n*-butyl alcohol, and 22 g (0.33 mole) of NaN_3 was heated to reflux for 4 days. The mixture was cooled and an additional 5 g of NaN_3 and 10 g of acetic acid was added and heating under reflux was continued for 2 additional days. After cooling, 300 ml of water was added and the *n*-butyl alcohol was removed *in vacuo*. Purification was achieved by way of the copper tetrazole derivative. Addition of a solution of 21.9 g of copper acetate in 200 ml of water precipitated the copper salt. After washing with water, the salt was suspended in 400 ml of water and H_2S was bubbled in until the precipitation of CuS was complete. The clear aqueous filtrate, after removing the CuS, was concentrated to dryness *in vacuo*. There was obtained 15.8 g of crude 5-(3-pyridylmethyl)tetrazole. Addition of a solution of ethyl acetate saturated with dry HCl to an ethanol solution of this product gave the salt, 17.5 g (40% yield), mp 188–190° dec. An analytical sample was prepared by a recrystallization from methanol–ether, mp 192–193° dec.

Anal. Calcd for $C_7H_8ClN_5$: C, 42.54; H, 4.08; N, 35.44; Cl, 17.94. Found: C, 42.54; H, 4.19; N, 35.42; Cl, 17.96.

1-Methyl-5-(3-pyridyl)tetrazole.—A mixture of 6.8 g (0.05 mole) of N-methylnicotinamide[45] and 10.4 g (0.05 mole) of PCl_5 in 125 ml of benzene was stirred at 25° for 6.5 hr. After this time, 30 ml of a solution of 4 *N* hydrazoic acid in benzene was added and stirring at 25° was continued for an additional 12 hr. After decanting the benzene from the insoluble gum that had formed, 50 ml of a dilute aqueous NaOH solution was added, and the product was extracted (CH_2Cl_2). Removal of the methylene chloride *in vacuo* gave 6.1 g of crude product, mp 66–72°. Recrystallizations from CH_2Cl_2–ether and from toluene gave 2.9 g (36% yield) of purified product, mp 78–80°. The analytical sample was prepared by sublimation, mp 78–80°.

2-Methyl-5-(3-pyridyl)tetrazole.—A suspension of 2.94 g (0.02 mole) of 5-(3-pyridyl)tetrazole and 1.23 ml (0.02 mole) of CH_3I in 40 ml of acetone was treated with a solution of 2.4 g (0.06 mole) of NaOH in 4 ml of water. The mixture was stirred and heated under reflux for 3 hr, filtered, diluted with 20 ml of water, and extracted with benzene. The organic layer was dried and concentrated *in vacuo*. Addition of 25 ml of water to the residue gave 0.97 g (30% yield) of product, mp 127–130°. The analytical sample was prepared by sublimation, mp 127.5–129°.

Pharmacology. Inhibition of FFA Release from Isolated Adipose Tissue.—The inhibition of norepinephrine-induced release of fatty acids was studied with epididymal adipose tissue taken from male Sprague–Dawley rats, 180–240 g, fed *ad libitum*. The tissue was placed in freshly aerated Krebs–Ringer bicarbonate buffer, pH 7.4, and minced with scissors into pieces weighing approximately 10 mg. Each experimental flask contained 3 ml of freshly aerated (95% O_2–5% CO_2) Krebs–Ringer bicarbonate buffer and 200 ± 3 mg (mean ± standard deviation) of adipose tissue. Bovine plasma albumin, fraction IV, 1%, was used as a fatty acid acceptor in the incubation medium. Adequate norepinephrine (20–30 ng/ml) was added to the incubation mixture to elicit a 50% of maximum fatty acid release. The compounds under test were added at appropriate concentrations. The experimental flasks were stoppered, aerated with 95% O_2–5% CO_2 for 10 min and incubated at 37° for 3 hr on a Dubnoff metabolic shaker. After incubation, aliquots were removed for fatty acid analysis by the method of Dole.[46] The effects of the inhibitors were expressed in terms of the molar concentration required to produce 50% inhibition (IC_{50}).

Effect on Fasting Plasma FFA.—An intravenous dose of 10 mg/kg of test compound was administered to two or more normal, fasted dogs. Blood samples were withdrawn for controls and at 0.5, 1 hr, and hourly through 8 hr. Plasma FFA levels were measured by the method of Dole[46] and are expressed as microequivalents of FFA per liter of plasma. A depression of plasma FFA with an intensity greater than 60% is classified as a maximal reduction (+++), a 30–60% depression is classified as a less than maximal reduction (++), and those compounds which produce less than a 30% fall of plasma FFA or are inactive are grouped together (±).

Acknowledgments.—The apparent ionization constants were determined by Mr. Thomas J. Toolan of the Physical Measurements Laboratory. The authors are grateful for the assistance of Messrs. Faustas J. Rajeckas, Richard Adams, and Albert Hamler in the preparation and Mrs. Dixie L. Wilson and Messrs. Gerald A. Mears and Dwight P. MacDonald in the pharmacological evaluation of these compounds. We also would like to acknowledge the many helpful discussions with Drs. J. M. McManus, F. A. Hochstein, and E. R. Pinson, Jr.

(43) R. P. Mariella and E. P. Belcher, *J. Am. Chem. Soc.*, **74**, 4049 (1952).

(45) Aldrich Chemical Co., Inc., Milwaukee, Wis.

(46) V. P. Dole, *J. Clin. Invest.*, **35**, 150 (1956).

The Antifertility Activity of Isoflavones Related to Genistein

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A group of 35 isoflavones has been synthesized by known procedures. They were tested for antifertility effects in a mouse litter prevention assay and as hypocholesteremic agents in normal rats.

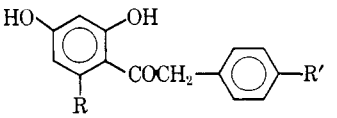
Although estrogens alone are effective antifertility agents,[1] they are employed either in combination with a progestin or sequentially, *i.e.*, followed by a progestin. The chronic administration of small doses of estrogen delays bleeding until 7–10 days following cessation of therapy, while chronic administration of larger doses of estrogen leads to irregular bleeding or spotting due to endometrial hyperplasia.[2] It has recently been re-

(1) A. S. Watnick, J. Gibson, M. Vinegra, and S. Tolksdorf, *Proc. Soc. Exptl. Biol. Med.*, **116**, 343 (1964).

(2) H. W. Rodel and F. A. Kincl, *Acta Endocrinol. Suppl.*, 105 (1966).

TABLE I

DEOXYBENZOINS



Compd	R	R'	Mp, °C[a]	λ_{max},[b] mμ	ε	Activity A[c]	Activity B[d]	Ref
I	H	H	114–115	316	8,600	−/10	0/25	*e*
				280	14,400			
				232	9,100			
II	H	OCH_3	161.5–162.5	317	9,000	−/10	0/25	*f*
				278	15,700			
				217	23,500			
				213	23,800			
III	H	NO_2	295–297	280	23,000	−/10	0/25	*g*
IV	H	Cl	159.5–160	290	20,800	−/10	0/50	*h*
				225	16,600			
V	OH	H	164–165			−/10	0/25	*h*
VI	OH	OH	269–270	287	21,300	−/10	0/25	*i*
				225	22,800			
VII	OH	OCH_3	198–199	288	19,900	−/10	0/25	*e*, *i*
				225	23,000			
VIII	OH	NO_2	249–250	291	28,900	−/10	0/50	*j*
IX	OH	Cl	224–225	290	21,200	−/10	0/25	*h*
				223	23,000			
				220	22,700			
X	OH	F	199–200	290	19,600	−/10	0/25	
				225	15,300			
XI	H	F	149–150	317	8,600	−/10	0/25	
				279	14,300			
				232	8,600			
XII	CH_3	OCH_3	109–110	277	8,300	−/10	0/25	
				223	20,400			

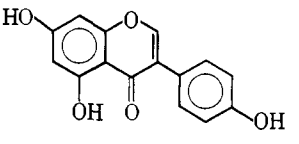
Compd	Crystn solvent	Formula	Calcd, % C	Calcd, % H	Calcd, % X	Found, % C	Found, % H	Found, % X
X	Aq methanol	$C_{14}H_{11}FO_4$	64.12	4.23	7.24	64.07	4.38	7.01
XI	Ether–hexane	$C_{14}H_{11}FO_3$	68.29	4.51	7.71	68.25	4.67	7.41
XII	Chloroform	$C_{16}H_{16}O_4$	70.57	5.92		70.66	6.07	

[a] Capillary tube, uncorrected. [b] In methanol. [c] Result in litter prevention in mice/dose (in milligrams per kilogram). [d] Percent lowering of serum cholesterol/dose (in milligrams per kilogram). [e] G. G. Badcock, G. W. R. Cavill, A. Robertson, and W. B. Whalley, *J. Chem. Soc.*, 2961 (1950). [f] W. Baker and F. M. Eastwood, *ibid.*, 2897 (1929). [g] P. C. Joshi and K. Venkataraman, *ibid.*, 513 (1934). [h] E. Chapman and H. Stephen, *ibid.*, 404 (1923). [i] W. Baker and R. Robinson, *ibid.*, 2713 (1926). [j] M. Yamashita, *Sci. Repl. Tokohu Imp. Univ., First Ser.*, **18,** 615 (1929); *Chem. Abstr.*, **24,** 2443 (1930).

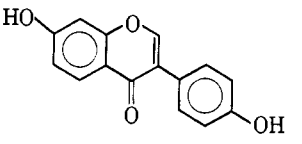
ported that ethinyl estradiol, a potent estrogen, is an effective postcoital agent, preventing normal blastocyst formation in rabbits by oral dosage 1–3 days following insemination.[3]

Such considerations have emphasized the examination of estrogen structures which might differentiate in some degree between gonadotrophin inhibition and endometrial hyperplasia, and which might also diminish some of the other biological properties of the known estrogens.

Warburton[4] has recounted the story of the 1941 outbreak of infertility in sheep in Western Australia. The responsible agent has since been identified as the predominant pasture, a subterranean clover containing large amounts of the isoflavones, genistein (I) and formononetin (II), in its leaves. Bradbury and White,[5] who isolated the isoflavones from fresh clover, found them to be very weakly estrogenic and suggested that



I



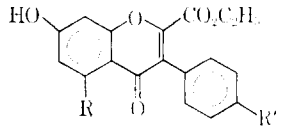
II

the isoflavones might be "proestrogens" to the much more potent isoflav-3-enes. This proposal has since been tested by Batterham, *et al.*,[6] in sheep rumen liquor incubations of biochanin A and of formononetin. No evidence of conversion to the isoflav-3-ene was found. It thus appeared possible that the antifertility effect of the isoflavones might be a property of the isoflavone structure, rather than dependent upon the low order of estrogenicity.[7]

(3) M. C. Chang and M. J. K. Harper, *Endocrinology*, **78**, 860 (1966).
(4) W. K. Warburton, *Quart. Rev.* (London), **8**, 83 (1954).
(5) R. B. Bradbury and D. J. White, *J. Chem. Soc.*, 3447 (1951).
(6) T. J. Batterham, N. K. Hart, J. A. Lamberton, *Nature*, **206**, 509 (1965).
(7) Recently, and since this work was completed, Y. Folman and G. S. Pope [*J. Endocrinol.*, **34**, 215 (1966)] have reported that the weak estrogens, such as coumestrol and genistein, exhibit an antiestrogenic effect against the potent estrogens, estrone, estradiol, and diethylstilbestrol, in terms of uterine and vaginal growth in immature mice.

Table II

2-Carbethoxyisoflavones



Compd	R	R'	Mp, °C[a]	λ_{max},[b] mμ	ε	Activity[c]		
						V[c]	B[d]	Ref
Ib	H	H	213–215	308	11,000	−/10	0/25	e
				237	24,000			
IIb	H	OCH_3	213.5–214.5	311	10,800	±/10	0/50	e
				240	24,800			
IIIb	H	NO_2	213–215	308	14,000	/10	0/25	e
				279	18,000			
				243	18,300			
IVb	H	Cl	211–212	311	10,800	−/10	0/25	
				239	26,400			
Vb	OH	H	233.5–234.5	312	6,400	−/10	0/25	e
				268	19,700			
VIIb	OH	OCH_3	191–191.5	307	8,200	/10	0/25	e
				267	24,200			
VIIIb	OH	NO_2	191–192	279	23,400	−/10	0/25	e
IXb	OH	Cl	195–197	308	7,250	±/10	0/25	
				270	22,000	−/5		
Xb	OH	F	204–206	313	10,800	−/10	0/25	
				268	19,900			
XIb	H	F	209–211	310	5,900	−/10	0/25	
				238	63,200			

Compd	Crystn solvent	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
IVb	Ethanol	$C_{18}H_{13}ClO_4$	62.71	3.80	10.29	62.64	4.03	10.52
IXb	Abs ethanol	$C_{18}H_{14}ClO_6$	59.92	3.63	9.83	60.04	3.74	9.72
Xb	Abs ethanol	$C_{18}H_{13}FO_6$	62.79	4.10	5.52	62.61	3.94	5.50
XIb	Ethanol	$C_{18}H_{13}FO_5$	65.85	3.99	5.79	65.93	4.09	5.53

[a–d] See corresponding footnote in Table I. [e] See ref 14.

A group of isoflavones was prepared by generally well-known procedures and tested in a litter-prevention assay in mice.[8] The compounds were administered orally daily and were considered active only if no pregnancies ensued following mating. The test results are presented in the tables (A) as + or −/dose. A test for the lowering of serum cholesterol[9] by oral dosage in normal male rats was also employed. The test results are expressed (B) as % lowering/dose. Only low orders of activity were found for any of the compounds.

The ultraviolet spectra of *trans*-stilbenes show a hypsochromic shift and decreased absorption for the long-wavelength band accompanying substitution at the α,α' positions, and this has been suggested as relating to estrogenic activity and to a twisting of the two aryl systems from the coplanar resonating structure.[10,11] A similar effect has been noted for 4-substituted isoflavenes,[12] and Lawson[13] has found high estrogenicity for 2,4-dialkyl-substituted isoflavenes.

While Bradbury and White[12] have shown that 2-alkyl substitution reduces estrogenic activity of the isoflavones, it was found here that no significant shift in the ultraviolet absorption could be assigned to alkyl substitution in position 2. In the absence of the 5-hydroxyl (compare IIe and XIV), the band shifts were slight with little diminution in absorption. Introduction of a hydroxyl group at position 5 produced a significant loss in absorption for the longer wavelength band, but this would more probably be an effect on the contribution of the 2,4-dihydroxyacetophenone system rather than an effect on the coplanarity of the 3-aryl system. Thus, steric inhibition of resonance by 2-alkyl substitution in the isoflavones is not readily identifiable in the ultraviolet spectra.

Experimental Section

Preparation of Benzyl Ketones.—The general procedure of treating the substituted resorcinol with an appropriately substituted phenylacetonitrile in ether in the presence of $ZnCl_2$ and anhydrous HCl was used (see Table I for references).

2-Carbethoxyisoflavones.—The procedure of Baker, *et al.*,[14] was employed.

Preparation of Isoflavones.—The 2-carbethoxyisoflavones were hydrolyzed and decarboxylated as previously described.[9] The 2-carboxyisoflavones were not purified but were decarboxylated directly.

(8) H. A. DeWald, O. D. Bird, G. Rodney, D. H. Kaump, and M. L. Black, *Nature*, **211**, 538 (1966).

(9) G. Rodney, M. L. Black, and O. H. Bird, *Biochem. Pharmacol.*, **14**, 445 (1965).

(10) W. H. Laarhoven, R. J. F. Nivard, and E. Havenga, *Rec. Trav. Chim.*, **79**, 1153 (1960).

(11) R. E. Juday, D. P. Page, and G. A. DuVall, *J. Med. Chem.*, **7**, 519 (1964).

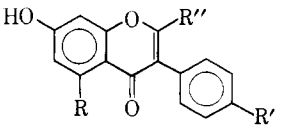
(12) R. B. Bradbury and D. E. White, *J. Chem. Soc.*, 871 (1953).

(13) W. Lawson, *ibid.*, 4448 (1954).

(14) W. Baker, A. Charlesworth, J. B. Harborne, and W. D. Ollis, *ibid.*, 1852 (1953).

TABLE III

ISOFLAVONES



Compd	R	R′	R″	Mp, °C[a]	λ_{max},[b] mμ	ε	Activity A[c]	Activity B[d]	Ref
Ic	H	H	H	213–215	300	11,400	−/10	0/25	e
					243	26,400			
IIc	H	OCH_3	H	257–258	301	11,100	−/10	0/25	e
					249	28,500			
IIIc	H	NO_2	H	292–293	297	23,200	±/10	15/25	e
					249	15,000	−/5		
					220	24,800			
IVc	H	Cl	H	261.5–262	300	11,000	−/10	0/25	f
					250	29,500			
Vc	OH	H	H	199–200	306	5,300	−/10	12/25	c
					258	28,800			
VIIc	OH	OCH_3	H	211.5–213	327	3,520	−/10	0.25	c
					261	35,300			
VIIIc	OH	NO_2	H	300–300.5	319[g]		−/10		e
					290	21,400			
					243	18,700			
IXc	OH	Cl	H	236–237	319[g]		−/10	0.25	
					261	33,600			
Xc	OH	F	H	224–225			−/10	0.25	
XIc	H	F	H	249–250.5	300	11,000	−/10	0/25	
					242	26,400			
XIII	H	OCH_3	CH_3	286–287	297	13,000	−/10	12/25	h
					248	27,900			
					241	28,500			
XIV	H	OCH_3	CH_2CH_3	242–243.5	297	13,300	−/10	13/25	
					248	27,300		15/50	
					241	28,300			
					233	27,400			
XV	H	OCH_3		268–269	310	16,000	−/10	14/25	
					237	29,000		15 '50	
					320	5,750	−/10	0/25	h
XVI	OH	OH	CH_2CH_3	245–246	288	8,750			
					257	31,600			
					320	4,200	−/10	0/25	h
XVII	OH	OCH_3	CH_2CH_3	229–230	290	6,000			
					257	32,000			
					293	12,900	−/10	0/25	
XVIII	CH_3	OCH_3	CH_2CH_3	238–239	250	28,900			
					242	29,300			

Compd	Crystn solvent	Formula	Calcd, % C	Calcd, % H	Calcd, % X	Found, % C	Found, % H	Found, % X
IXc	Ethyl acetate	$C_{15}H_9ClO_4$	62.40	3.14	12.28	62.30	3.01	12.25
Xc	Ether	$C_{15}H_9FO_4$	66.17	3.33	6.98	66.14	3.41	6.92
XIc	Acetone	$C_{15}H_9FO_3$	70.31	3.54	7.41	70.23	3.73	7.16
XIV	Methanol	$C_{18}H_{16}O_4$	72.96	5.44		73.08	5.47	
XV	Acetone–methanol	$C_{22}H_{16}O_4$	76.73	4.68		76.81	4.75	
XVIII	Ethyl acetate	$C_{19}H_{18}O_4$	73.52	5.84		73.38	5.98	

[a–d] See corresponding footnote in Table I. [e] See ref 14. [f] L. Farkas, A. Major, L. Pallos, and J. Varady, *Chem. Ber.*, **91**, 2858 (1958). [g] Inflection. [h] See ref 12.

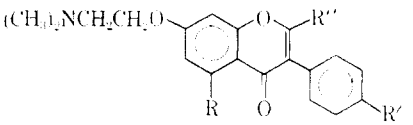
Preparation of 7-Dimethylaminoethoxyisoflavones.—The 7-hydroxyisoflavone (1 mole) in absolute ethanol was treated with 2.2 moles of KOH in water and then with 1.1 moles of dimethylaminoethyl chloride hydrochloride. The reaction mixture was refluxed and stirred for 1–2 hr, cooled, and diluted with water. The precipitate was separated by filtration and washed with water. Unreacted starting material could be recovered by acidification of the filtrate.

In other preparations, NaH was used in diglyme solution. After the reflux period, the reaction mixture was poured into water to precipitate the product. Data on the novel members of each of these groups of compounds are presented in Tables I–IV (Table IV may be found on the following page).

Acknowledgment.—The authors wish to express their appreciation to Dr. L. M. Long for encouragement in this investigation and to Drs. O. D. Bird and G. Rodney for the assay results. We thank Dr. J. M. Vandenbelt and co-workers for the physical data and Mr. C. E. Childs and staff for the microanalyses.

TABLE IV

DIALKYLAMINOALKOXYISOFLAVONES



Compd	R	R′	R″	Mp, °C[a]	λ_{max}^{EtOH}, mμ	ε	Activity[b] IV	Activity[b] IV′
XIX[c]	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		
					244	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[d]			
					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	Calcd, % H	Calcd, % N	Calcd, % X	Found, % C	Found, % H	Found, % N	Found, % X
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	5.94
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	$C_{28}H_{27}NO_4$	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 Sipher, S. A., Belgian Patent 635541 (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. Linsmaier and E. Corday, *J. Am. Med. Assoc.*, **171**, 1098 (1959).