The Effect of Substituted Carboxylic Acids on Hepatic Cholesterogenesis[1]

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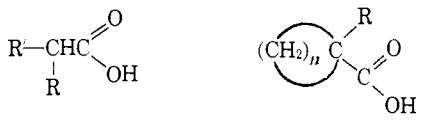
Substituted cycloalkane carboxylic acids, $(CH_2)_nC<^{R}_{COOH}$ (R = 4-biphenylyl or 2-fluorenyl), having an alicyclic sytem ranging from cyclopropane to cyclohexane were synthesized. The synthetic methods employed were (a) condensation of the corresponding aryl acetonitrile with the appropriate α,ω-dibromide of the hydrocarbon and (b) quasi-Favorskiĭ rearrangement of the corresponding aralkyl bromo ketone under strongly basic conditions. When 4-biphenylyl-1-bromocyclopentyl ketone (VIIa) was treated under quasi-Favorskiĭ conditions, an "abnormal" product was obtained. The structure of this compound was assigned based on its spectral properties and confirmed by unequivocal synthesis. While some of the substituted carboxylic acids inhibited cholesterogenesis from mevalonate in rat liver homogenates, none exhibited any antihypercholesterolemic effect in nephrotic rats.

The first observation that phenylacetic acid derivatives might be useful as cholesterol lowering agents was made by Cottet, *et al.*[3] They claimed that α-phenylbutyric acid (Ia) was clinically effective in the treatment of hypercholesterolemia. These results were not confirmed by other workers.[4,5] Steinberg and his collaborators[6,7] and Garattini, *et al.*,[8] have shown that the compound exerts its inhibitory effect at the acetate activation stage. Possible interference with fatty acid metabolism coupled with the weak antihypercholesterolemic activity render this agent of doubtful therapeutic value.

A definite advance in the direction of producing potent and more selective hypocholesterolemic agents was revealed by the following findings. When the α-phenyl group was replaced by a 4-biphenylyl moiety, the compounds produced were more active and caused appreciable inhibition in the cholesterol biosynthetic pathway at some post-mevalonate step.[9,10] 2-(4-Biphenylyl)-4-hexenoic acid[11](Ib) is a highly active member of this class and has been shown to be active *in vivo* as an antagonist to the first phase of Triton-induced hypercholesterolemia in the rat[12] as well as an antagonist to dietary hypercholesterolemia in dogs.[13] Results in the clinic with 2-(4-biphenylyl)-4-hexenoic acid (Ib) have been variable.[14,15]

In an attempt to obtain compounds having specific hypocholesterolemic activity inhibiting the biosynthesis at a post-mevalonate stage, substituted carboxylic acids and carboxylic acid derivatives of the type II (R = 4-biphenylyl or 2-fluorenyl) were synthesized. These compounds were prepared by the two general methods shown in Chart I.

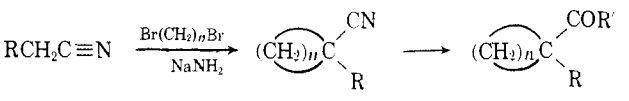


Ia, R = phenyl; R′ = ethyl
b, R = 4-biphenylyl; R′ = $CH_2CH{=}CHCH_3$

II, R = 4-biphenylyl or 2-fluorenyl

CHART I

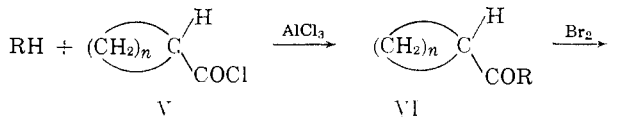
METHOD A



III IV

IIIa, R = 4-biphenylyl; n = 2
b, R = 4-biphenylyl; n = 3
c, R = 4-biphenylyl; n = 4
IVa, R = 4-biphenylyl; R′ = OH; n = 2
b, R = 4-biphenylyl; R′ = OH; n = 3
c, R = 4-biphenylyl; R′ = OH; n = 4
d, R = 4-biphenylyl; R′ = NH_2; n = 4

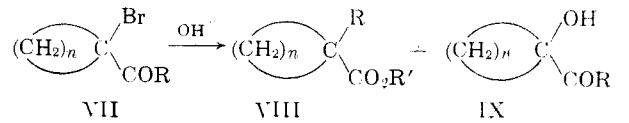
METHOD B



V VI

a, n = 4
b, n = 5

a, R = 4-biphenylyl; n = 4
b, R = 4-biphenylyl; n = 5
c, R = 2-fluorenyl; n = 5



VII VIII IX

a, as in VI
b, as in VI
c, as in VI

a, R = 4-biphenylyl; R′ = H; n = 5
b, R = 2-fluorenyl; R′ = H; n = 5
c, R = 4-biphenylyl; R′ = $CH_2CH_2N(C_2H_5)_2$; n = 5

(1) Part V of a series entitled "Agents Affecting Lipid Metabolism." For part IV *cf.* D. Dvornik, M. Kraml, J. Dubuc, M. Givner, and R. Gaudry, *J. Am. Chem. Soc.*, **85**, 3309 (1963).

(2) Author to whom inquiries should be addressed: Department of Chemistry, University of Ottawa, Ottawa, Canada.

(3) J. Cottet, A. Mathivat, and J. Redel, *Presse Med.*, **62**, 939 (1954).

(4) D. S. Fredrickson and D. Steinberg, *Circulation*, **15**, 391 (1957).

(5) F. Grande, J. T. Anderson, and A. Keys, *Metab. Clin. Exptl.*, **6**, 154 (1957).

(6) D. Steinberg and D. S. Fredrickson, *Proc. Soc. Exptl. Biol. Med.*, **90**, 232 (1954).

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(8) S. Garattini, C. Morpurgo, B. Morelli, R. Paoletti, and N. Passerini, *Arch. Intern. Pharmacodyn.*, **109**, 400 (1957).

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(13) M. Massaioli, O. Cennamo, G. Balbo, and N. Masera, *Boll. Soc. Piemontese Chir.*, **30**, 3 (1960).

(14) E. Teslori, *Gazz. Intern. Med. Chir.*, **63**, 2447 (1959).

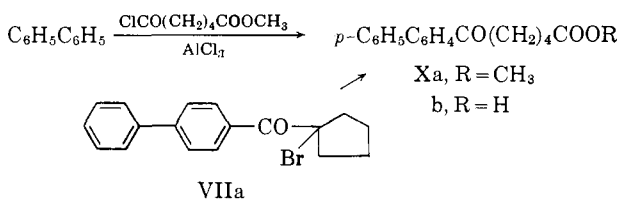
(15) E. Sabbadini and M. Gazzaniga, *Minerva Med.*, **40**, 1543 (1959).

1-(4-Biphenylyl)-1-cyanocyclopropane (IIIa), -cyclobutane (IIIb), and -cyclopentane (IIIc) were prepared[16] by the treatment of ethylene dibromide, 1,3-dibromopropane, and 1,4-dibromobutane, respectively, with the disodium salt of *p*-phenylbenzyl cyanide anion.[17] Hydrolysis of the resulting nitriles gave the corresponding carboxylic acids (IVa–c). The hydrolysis of IIIc also gave amide IVd in addition to the acid IVc.

An alternative route to the desired carboxylic acids involved the corresponding aralkyl ketones (VIa–c). These ketones were obtained by Friedel–Crafts reaction of the acid chlorides of the appropriate alicyclic carboxylic acids with the aromatic hydrocarbons. The bromo derivatives of these ketones underwent quasi-Favorskiĭ[18] rearrangement on treatment with powdered sodium hydroxide in boiling xylene. In the case of 4-biphenylyl-1-bromocyclohexyl ketone (VIIb) the desired 1-(4-biphenylyl)cyclohexanecarboxylic acid[19] (VIIIa, 34%) was obtained with the concomitant formation of a large amount (43%) of 4-biphenylyl-1-hydroxycyclohexyl ketone[18b] (IXa). The bromo ketone (VIIc) having a 2-fluorenyl substituent gave a poorer yield of the corresponding carboxylic acid (VIIIb) in the quasi-Favorskiĭ reaction. Here again a substantial amount of hydroxy ketone (IXb) was isolated.

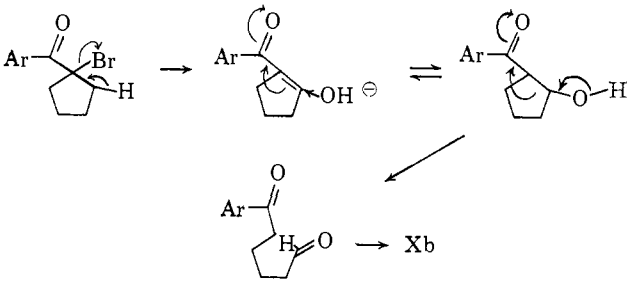
When 4-biphenylyl-1-bromocyclopentyl ketone (VIIa) was treated under quasi-Favorskiĭ conditions[18] none of the expected 1-(4-biphenylyl)cyclopentanecarboxylic acid could be isolated. Instead, a compound was obtained in 54% yield which absorbed at 286 mμ (ε 20,600) in ultraviolet light (expected absorption would be at 256 mμ) and exhibited bands at 1680, 1700 (C=O absorption) and in the region 3500–2500 (COOH) cm.$^{-1}$ in its infrared spectrum. Based on the spectral and analytical data this product was identified as 6-oxo-6-(4-biphenylyl)hexanoic acid (Xb).

This structural assignment was confirmed by an unequivocal synthesis of Xb. When 5-carbomethoxyvaleryl chloride was treated with biphenyl in the presence of aluminum chloride, the keto ester Xa was isolated. Hydrolysis of this substance gave an acid which was identical in all respects with the keto acid Xb obtained from the reaction of 4-biphenylyl-1-bromocyclopentyl ketone (VIIa) under conditions[18] for quasi-Favorskiĭ rearrangement.



VIIa

The mode of formation of acid Xb is worthy of comment. One possible pathway to this product is depicted below. It appears that the rate of quasi-Favorskiĭ rearrangement in the cyclopentyl series must be much slower relative to that in the case of the cyclohexyl compound, to allow the formation of compound Xb in over 50% yield. It is probable that these rate differences result from the inherent stereochemical differences between the five- and six-membered ring systems.



Biological Methods. A. Cholesterol Biosynthesis Inhibition.—The ability of the test compounds to inhibit the incorporation of mevalonate-2-C^{14} into cholesterol by rat liver homogenates was estimated as previously described.[20]

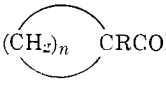
B. Antihypercholesterolemic Effect.—This effect was measured in rats that had been made hypercholesterolemic by inducing nephrosis *via* daily administration of the aminonucleoside of puromycin[21] (17.5 mg./kg. s.c.) for 8 days. Test compounds were administered daily (s.c.) from day 3 to 12 and their ability to prevent the rise in serum cholesterol was taken as a measure of their antihypercholesterolemic effect. Serum cholesterol levels were measured using the method of Pearson, *et al.*[22]

Results and Discussion

The results of *in vitro* inhibition are presented in Table I. 2-(4-Biphenylyl)-4-hexenoic acid (Ib), our reference compound, inhibited cholesterol biosynthesis 91% at $1 \times 10^{-3} M$ final but was inactive at $1 \times 10^{-4} M$. In our series of compounds only three (Xb, VIIIa, and VIIIc) were as potent as Ib, inhibiting 86, 94, and 98%, respectively, at $1 \times 10^{-3} M$ final. At $0.5 \times 10^{-4} M$ final, VIIIc was devoid of activity.

TABLE I

EFFECT ON INCORPORATION OF MEVALONATE-2-C^{14} INTO CHOLESTEROL BY RAT LIVER HOMOGENATES



No.	R	R'	n	% inhibition at $1 \times 10^{-3} M$, final
IVa	4-Biphenylyl	OH	2	21
IVb	4-Biphenylyl	OH	3	7
IVc	4-Biphenylyl	OH	4	0
IVd	4-Biphenylyl	NH_2	4	0
VIIIa	4-Biphenylyl	OH	5	94
VIIIc	4-Biphenylyl	$OCH_2CH_2N(C_2H_5)_2$	5	98 (0 at $0.5 \times 10^{-4} M$)
VIIIb	2-Fluorenyl	OH	5	0
	Miscellaneous Compounds			
Xb	p-$C_6H_5C_6H_4CO(CH_2)_4COOH$			86
Xa	p-$C_6H_5C_6H_4CO(CH_2)_4COOCH_3$			32
Ib	p-$C_6H_5C_6H_4CHCH_2CH{=}CHCH_3$ (COOH)			91 (0 at $1.0 \times 10^{-4} M$)

Compound VIIIa, being among the most active in the series *in vitro* and being available in sufficiently large amounts, was tested as an antihypercholesterolemic agent in rats rendered nephrotic and hyper-

(16) F. H. Case, *J. Am. Chem. Soc.*, **56**, 715 (1934).

(17) (a) R. Lesser, *Chem. Abstr.*, **32**, 4798 (1938); German Patent 658,114 (1938); (b) G. Cavallini and E. Massarani, Italian Patent 600,214; *Chem. Abstr.*, **55**, 16,486 (1961).

(18) (a) *Cf.* C. L. Stevens and E. Farkas, *J. Am. Chem. Soc.*, **74**, 5352 (1952); (b) E. E. Smissman and G. Hite, *ibid.*, **81**, 1201 (1959).

(19) N. H. Cromwell and P. H. Hess, *ibid.*, **82**, 136 (1960).

(20) L. G. Humber, M. Kraml, and J. Dubuc, *Biochem. Pharmacol.*, **11**, 755 (1962).

(21) C. Chappel and K. Voith, private communication.

(22) S. Pearson, S. Stern, and T. H. McGavack, *Anal. Chem.*, **25**, 813 (1953).

TABLE II

CHOLESTEROL-LOWERING EFFECT IN HYPERCHOLESTEROLEMIC NEPHROTIC RATS[a]

Compound (R = 4-biphenylyl)		Dose	Cholesterol mg./100 ml.[b]	% reduction	Significance
		0	741 ± 57[c]	...	...
Ib	$CH_3CH{=}CHCH_2CHRCOOH$	40 mg./kg.	550 ± 35	26	$0.01 < P < 0.02$
		20 mg./kg.	611 ± 45	18	$0.05 < P < 0.10$
VIIIa		0	612 ± 51	...	...
		25 mg./kg.	653 ± 28	0	None

[a] Rats were made nephrotic by daily s.c. administration of 17.5 mg./kg. of the aminonucleoside of puromycin for 8 days. Eight rats were used in each test group. [b] Standard error.

cholesterolemic in the manner described above. 2-(4-Biphenylyl)-4-hexenoic acid was the reference compound and the results are presented in Table II. As can be seen, the control animals are markedly hypercholesterolemic, the levels having risen to the 600–700 mg./100 ml. range from a normal of approximately 100 mg./100 ml. 2-(4-Biphenylyl)-4-hexenoic acid was active at both the 40 and 20 mg./kg. dosage schedules, dropping cholesterol levels 26 and 18%, respectively. However VIIIa was inactive in this *in vivo* test.

While some compounds in this series are active *in vitro* as cholesterol biosynthesis inhibitors, this activity is not reflected *in vivo* as evidenced by the failure of VIIIa to depress elevated cholesterol levels. Compounds of the type $(CH_2)_nCRCOOR'$, as exemplified by our series, are less active than 2-(4-biphenylyl)-4-hexenoic acid (Ib).

Experimental[23]

4-Biphenylyl Cyclopentyl Ketone (VIa).—A solution of cyclopentanecarbonyl chloride (Va, 24.9 g.) and biphenyl (26.5 g.) in dry carbon disulfide (80 ml.) was added over a period of 30 min. to a stirred suspension of anhydrous aluminum chloride (26.5 g.) in carbon disulfide (80 ml.) at 0°. The reaction was quenched by pouring the mixture into water and ice containing concentrated hydrochloric acid. This was extracted with ether and the extracts were washed with dilute sodium bicarbonate solution and then water. The ether solution was dried and evaporated to dryness leaving an oily solid (41.2 g.) which, after crystallization from ethanol, had m.p. 56–78°, λ_{max} 283 mμ (ε 23,000). The crude product could be used without further purification for the next step. An analytical sample prepared by treatment with Girard's reagent[24] had m.p. 61–63°; λ_{max} 283 mμ (ε 25,000); $\nu_{max}^{CS_2}$ 1680 cm.$^{-1}$.

Anal. Calcd. for $C_{18}H_{18}O$: C, 86.58; H, 7.25. Found: C, 86.44; H, 7.23.

4-Biphenylyl 1-Bromocyclopentyl Ketone (VIIa).—Crude 4-biphenylyl cyclopentyl ketone (VIa, 10.0 g.) was dissolved in acetic acid (91 ml.) and heated to 85°. Bromine (6.4 g.) was added and the solution decolorized immediately. More bromine was then added until a faint yellow color persisted and the solution was kept at 85° for 30 min. The cooled solution was poured into cold water and the gummy material which precipitated was extracted with ether. After washing the extracts with sodium bicarbonate and water, the ether solution was dried and the solvent removed. The brown sirup obtained was chromatographed on Florisil[23] (300 g.). Elution with petroleum ether gave biphenyl (2.01 g.) and on changing to petroleum ether–benzene (4:1) the bromo ketone (3.67 g.) was obtained. One crystallization from ethanol gave an analytical sample, m.p. 87–88°; λ_{max} 295 mμ (ε 23,400); $\nu_{max}^{CS_2}$ 1676 cm.$^{-1}$).

Anal. Calcd. for $C_{18}H_{17}BrO$: C, 65.66; H, 5.21; Br, 24.28. Found: C, 65.40; H, 5.31; Br, 23.96, 24.16.

1-(4-Biphenylyl)cyclohexanecarboxylic Acid (VIIIa).—4-Biphenylyl 1-bromocyclohexyl ketone[19] (15.0 g., m.p. 113–114°), dissolved in dry xylene (75 ml.), was added over a period of 20 min. to a stirred, refluxing mixture of dry xylene (600 ml.) and powdered sodium hydroxide[18b] (52.0 g.). The reaction mixture was stirred and refluxed for 2 hr., then cooled and washed with water until neutral. The aqueous layer was acidified with hydrochloric acid and extracted with ether. After washing the extracts thoroughly with water, the solution was dried and the solvent was removed leaving the crude acid (4.2 g.). One crystallization from benzene gave the pure acid, m.p. 175–179°; λ_{max} 256 mμ (ε 24,700); $\nu_{max}^{CS_2}$ 1691 cm.$^{-1}$ (lit.[19] m.p. 177–178°).

The neutral xylene solution above was dried and evaporated to dryness. Crystallization of the residue from benzene gave a first (3.82 g.) and a second crop (1.44 g.) of a substance identified as **4-biphenylyl 1-hydroxycyclohexyl ketone (IXa).** An analytical sample melted at 116–117°; λ_{max} 286 mμ (ε 25,200); $\nu_{max}^{CS_2}$ 3610 (bonded O–H), 3470 (nonbonded O–H), 1675, 1662 (carbonyl group adjacent to aromatic ring) cm.$^{-1}$).

Anal. Calcd. for $C_{19}H_{20}O_2$: C, 81.39; H, 7.19. Found: C, 81.49; H, 7.05.

β-Diethylaminoethyl 1-(4-Biphenylyl)cyclohexanecarboxylate Hydrochloride (VIIIc).—2-Chlorotriethylamine hydrochloride (2.66 g.) was added to a solution of sodium methoxide (0.87 g.) in 2-propanol[25] (20 ml.). After heating for a few min. the solution was filtered and the filtrate was added to a solution of 1-(4-biphenylyl)cyclohexanecarboxylic acid (VIIIa, 4.20 g.) in 2-propanol (30 ml.). The reaction solution was refluxed overnight, and the volume of the solution was then reduced by half. Aqueous 5% sodium hydroxide was added to the solution which was then extracted with ether. Work-up in the usual way resulted in the isolation of an oily liquid which was taken up in methanolic hydrogen chloride solution and left standing overnight. Removal of the methanol left a greenish oil which solidified on standing. Crystallization from acetone afforded a first crop of fine needles (1.01 g.), m.p. 189–192°, and a second crop (0.83 g.), m.p. 188–191°. An analytical sample melted at 189–191°; λ_{max} 256 mμ (ε 24,800); $\nu_{max}^{(mull)}$ 1725 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{30}NO_2$·HCl: C, 72.18; H, 8.24; N, 3.37; Cl, 8.52. Found: C, 71.86; H, 8.54; N, 3.41; Cl, 8.34.

Quasi-Favorskiĭ Rearrangement of 4-Biphenylyl 1-Bromocyclopentyl Ketone (VIIa).—4-Biphenylyl 1-bromocyclopentyl ketone (VIIa, 2.8 g.), dissolved in dry xylene (15 ml.), was added slowly to a stirred, refluxing mixture of anhydrous xylene (100 ml.) and powdered sodium hydroxide[18b] (10 g.). Following the same procedure described in the preparation of 1-(4-biphenylyl)cyclohexanecarboxylic acid (VIIIa), a light brown amorphous substance (1.5 g.) was isolated, m.p. 156–164°. Several crystallizations from benzene gave a pure acid [m.p. 162–163°; λ_{max} 286 mμ (ε 20,600); ν_{max}^{mull} 1700 (carboxylic acid), 1680 (carbonyl group adjacent to aromatic ring) cm.$^{-1}$] identified as **6-oxo-6-(4-biphenylyl)hexanoic acid (Xb).**

Anal. Calcd. for $C_{18}H_{18}O_3$: C, 76.57; H, 6.43. Found: C, 76.38; H, 6.29.

6-Oxo-6-(4-biphenylyl)hexanoic Acid (Xb).—A solution of biphenyl (10.6 g.) and 5-carbomethoxyvaleryl chloride (12.3 g.) in dry carbon disulfide (60 ml.) was slowly added (30 ml.) to a stirred mixture of anhydrous aluminum chloride (12.0 g.) and

(23) Melting points were determined on a Thiele–Dennis apparatus and are corrected. Infrared spectra were recorded on a Perkin-Elmer (Model 21) spectrophotometer equipped with sodium chloride optics. Ultraviolet spectra were taken in ethanol with a Beckman (Model DK) recording instrument. Florisil 160–100 mesh, Floridin Co.) and silica gel (Davison, grade 923, 100–200 mesh) were used for chromatography. Petroleum ether refers to the fraction with b.p. 30–60°. Organic extracts were dried over anhydrous magnesium sulfate and the solvents were removed under vacuum.

(24) L. F. Fieser in "Experiments in Organic Chemistry," D. C. Heath and Company, Boston, Mass., 1957, p. 88.

(25) Cf. C. H. Tilford, M. G. Van Campen, Jr., and R. S. Shelton, *J. Am. Chem. Soc.*, **69**, 2902 (1947).

carbon disulfide (60 ml.). The reaction mixture was cooled in an ice–water bath during the addition, and stirring was continued for 15 min. at room temperature. After pouring the reaction mixture into cold 1:3 hydrochloric acid–water, the layers were separated and the aqueous phase was extracted with ether. The carbon disulfide solution and ether extracts were combined and washed with 5% aqueous sodium hydroxide solution and water. A yellow solid (18.7 g.) was isolated after the organic solution had been dried and subsequently evaporated to dryness. An analytical sample of **methyl 6-oxo-6-(4-biphenylyl)hexanoate (Xa)** melted at 108–109°; λ_{max} 285 mμ (ε 23,900); $\nu_{max}^{CS_2}$ 1737 (ester carbonyl), 1685 (carbonyl adjacent to aromatic ring) cm.$^{-1}$, and was obtained after three crystallizations from acetone–hexane.

Anal. Calcd. for $C_{19}H_{20}O_3$: C, 77.00; H, 6.80. Found: C, 77.06; H, 6.86.

Methyl 6-oxo-6-(4-biphenylyl)-hexanoate (Xa, 1.0 g.) prepared above was dissolved in methanol (25 ml.) and 5% aqueous sodium hydroxide solution (5 ml.) was added. This solution was refluxed for 1 hr., cooled, diluted with water, and extracted with ether. The aqueous extract was acidified (hydrochloric acid) and then extracted with ether. Working up in the usual way gave a white solid (0.87 g.) which after one crystallization from benzene melted at 162–163°; λ_{max} 287 mμ (ε 26,150); ν_{max}^{mull} 1700 and 1680 cm.$^{-1}$. Comparison of infrared spectra and a mixture melting point showed that this compound (Xb) was identical with the acid obtained in the quasi-Favorskii rearrangement of 4-biphenylyl 1-bromocyclopentyl ketone (VIIa).

2-Fluorenyl Cyclohexyl Ketone (VIc).—A solution of fluorene (25.0 g.) and cyclohexanecarbonyl chloride (23.0 g.) in anhydrous carbon disulfide (80 ml.) was added over a period of 40 min. to a stirred mixture of anhydrous aluminum chloride (30.0 g.) and carbon disulfide (100 ml.) cooled in an ice–water bath. After the addition was completed, the cooling bath was removed and stirring was continued at room temperature for 30 min. Working up in the usual way gave a crude product which after one crystallization from methanol afforded needles (23.5 g.), m.p. 150–152°. A second crop (8.2 g.), m.p. 147–150°, was also obtained. An analytical sample melted at 151–152°; λ_{max} 297 (ε 23,900), 317 mμ (ε 26,350); $\nu_{max}^{CS_2}$ 1676 cm.$^{-1}$.

Anal. Calcd. for $C_{20}H_{20}O$: C, 86.92; H, 7.29. Found: C, 86.80; H, 7.15.

2-Fluorenyl 1-Bromocyclohexyl Ketone (VIIc).—2-Fluorenyl-cyclohexyl ketone (IIc, 10.0 g.) was dissolved in chloroform (100 ml.) and warmed gently while a solution of bromine (5.8 g.) in chloroform (40 ml.) was added over a period of 45 min. with stirring. The volume of the solution was concentrated, methanol was added, and the product crystallized as plates (11.0 g.), m.p. 118–124°. Two more crystallizations from chloroform–methanol gave an analytical sample, m.p. 121–122; λ_{max} 242 (ε 8570), 318 mμ (ε 25,000); $\nu_{max}^{CS_2}$ 1675 (carbonyl adjacent to aromatic ring) cm.$^{-1}$.

Anal. Calcd. for $C_{20}H_{19}BrO$: C, 67.60; H, 5.38; Br, 22.50. Found: C, 67.57; H, 5.61; Br, 22.75, 22.72.

Quasi-Favorskii Rearrangement of 2-Fluorenyl 1-Bromocyclohexyl Ketone (VIIc).—A solution of 2-fluorenyl 1-bromocyclohexyl ketone (VIIc, 9.5 g.) in dry xylene (45 ml.) was added to a suspension of powdered sodium hydroxide[18b] (34 g.) in refluxing xylene (400 ml.) over 20 min. with vigorous stirring. The reaction mixture was stirred and refluxed for 2 hr., cooled to room temperature, and washed with water until washings were neutral. A gummy material precipitated at the interface and was collected by filtration. This could now be dissolved in water and after washing the aqueous solution with ether it was acidified with hydrochloric acid. A red solid (2.2 g.) precipitated which was collected by filtration. The basic aqueous extracts above were found to contain only traces of acid. Chromatography of the red solid over silica gel[23] and elution with benzene–chloroform (24:1) gave a white solid (0.52 g.) which after 4 crystallizations from aqueous methanol gave analytically pure **1-(2-fluorenyl)-cyclohexanecarboxylic acid (VIIIb)**, m.p. 207–209°; λ_{max} 270 (ε 29,200), 295 (9380), 307 mμ (13,100); $\nu_{max}^{CS_2}$ 1692 (carboxylic acid) cm.$^{-1}$.

Anal. Calcd. for $C_{20}H_{20}O_2$: C, 82.15; H, 6.89. Found: C, 81.96; H, 6.92.

The neutral xylene solution above was dried and evaporated to dryness. One crystallization of the residue from benzene–hexane gave a substance (2.0 g.) identified as **2-fluorenyl 1-hydroxycyclohexyl ketone (IXb)**, m.p. 131–135°. An analytical sample had m.p. 140–141°; λ_{max} 314 mμ (ε 25,800); $\nu_{max}^{CS_2}$ 3610 (bonded O–H), 3470 (nonbonded O–H), 1671, 1657 (carbonyl adjacent to aromatic ring) cm.$^{-1}$.

Anal. Calcd. for $C_{20}H_{20}O_2$: C, 82.15; H, 6.89. Found: C, 82.43; H, 6.76.

1-(4-Biphenylyl)-1-cyanocyclopropane (IIIa).—*p*-Phenylbenzyl cyanide (15.0 g.) was dissolved in anhydrous ether (250 ml.) and sodamide (6.5 g.) was added to the solution. This was refluxed with stirring and after a short time a red color appeared which became very intense after 0.5 hr. At this point ethylene dibromide[16] (14.65 g.) dissolved in dry ether (20 ml.) was slowly added over a period of 15 min. Refluxing with stirring was continued for 9 hr. and then the reaction mixture was poured over crushed ice and extracted with ether. The combined extracts were washed with water, dried, and evaporated to dryness. A red solid (14.9 g.), m.p. 80–88°, was obtained which after several crystallizations from acetone–hexane afforded an analytical sample, m.p. 108°; λ_{max} 256 mμ (ε 25,800); $\nu_{max}^{CS_2}$ 2240 (nitrile group) cm.$^{-1}$.

Anal. Calcd. for $C_{16}H_{13}N$: C, 87.64; H, 5.98; N, 6.39. Found: C, 87.65, H, 6.09; N, 6.62.

1-(4-Biphenylyl)cyclopropanecarboxylic Acid (IVa).—1-(4-Biphenylyl)-1-cyanocyclopropane (IIIa, 5.0 g.) was dissolved in ethanol (70 ml.) and to this was added a solution of potassium hydroxide (20 g.) in water (20 ml.). The reaction mixture was refluxed for 20 hr., cooled, and diluted with water. After extraction with ether, the ether solution was in turn extracted with 5% aqueous sodium hydroxide solution. The first basic extract was a clear red solution but after a few sec. a white solid precipitated and was collected by filtration. This solid was dissolved in water and on acidification with dilute hydrochloric acid, **1-(4-biphenylyl)cyclopropanecarboxylic acid** (IVa, 1.97 g.), m.p. 215–219°, precipitated as a white solid. An analytical sample prepared by two crystallizations from aqueous ethanol was obtained as fine needles, m.p. 218–221°; λ_{max} 255 mμ (ε 24,300); $\nu_{max}^{CS_2}$ 1690 (carboxylic acid) cm.$^{-1}$.

Anal. Calcd. for $C_{16}H_{14}O_2$: C, 80.64; H, 5.92. Found: C, 80.79; H, 6.10.

1-(4-Biphenylyl)-1-cyanocyclobutane (IIIb).—*p*-Phenylbenzyl cyanide (15.0 g.), dissolved in dry ether (250 ml.), was condensed with 1,3-dibromopropane (15.8 g.) in the presence of sodamide (6.0 g.) under the same conditions[16] as those used in the preparation of IIIa. The reaction was quenched by pouring the mixture over crushed ice. An emulsion formed which was broken by filtration and the solid (5.5 g.) which was collected was found to be crude IIIb (nitrile band at 2240 cm.$^{-1}$ compared to 2265 cm.$^{-1}$ in *p*-phenylbenzyl cyanide). Separation of the layers in the filtrate and extraction of the aqueous layer with ether resulted in the isolation of a reddish brown oil (11.7 g.) which was also identified as crude IIIb. An analytical sample was obtained by chromatography on Florisil[23] and crystallization from acetone–hexane, m.p. 115–116°; λ_{max} 254 mμ (ε 22,400); $\nu_{max}^{CHCl_3}$ 2240 (nitrile group) cm.$^{-1}$.

Anal. Calcd. for $C_{17}H_{15}N$: C, 87.51; H, 6.48. Found: C, 87.64; H, 6.52.

1-(4-Biphenylyl)cyclobutanecarboxylic Acid (IVb).—Crude 1-(4-biphenylyl)-1-cyanocyclobutane (IIIb, 7.1 g.) was dissolved in ethanol (100 ml.) and this solution was added to a solution of potassium hydroxide (28.3 g.) in water (28 ml.). The reaction solution was refluxed for 20 hr., cooled, diluted with water and extracted with ether. Acidification of the aqueous solution with dilute hydrochloric acid precipitated a white solid (1.73 g.), m.p. 121–136°. One crystallization from aqueous methanol raised the melting point to 146–150°. An analytical sample melted at 150–154°; λ_{max} 246 mμ (ε 21,700); $\nu_{max}^{CS_2}$ 1695 (carboxylic acid) cm.$^{-1}$.

Anal. Calcd. for $C_{17}H_{16}O_2$: C, 80.92; H, 6.39. Found: C, 80.65; H, 6.32.

1-(4-Biphenylyl)-1-cyanocyclopentane (IIIc).—*p*-Phenylbenzyl cyanide (15.0 g.), dissolved in anhydrous ether (250 ml.), was condensed with 1,4-dibromobutane (16.85 g.) in the presence of sodamide (6.0 g.) under the same conditions[16] as those used in the preparation of IIIa. The crude product (18.9 g.) on crystallization from hexane gave a first crop (8.50 g.), m.p. 75–81°, and a second crop (3.47 g.). An analytical sample melted at 91–93°; λ_{max} 252 (ε 28,800), 258 mμ (29,200); $\nu_{max}^{CHCl_3}$ 2238 (nitrile group) cm.$^{-1}$.

Anal. Calcd. for $C_{18}H_{17}N$: C, 87.41; H, 6.93; N, 5.66. Found: C, 87.70; H, 7.04; N, 5.83, 5.77.

Hydrolysis of 1-(4-Biphenylyl)-1-cyanocyclopentane (IIIc).—Crude 1-(4-biphenylyl)-1-cyanocyclopentane (IIIc, 8.5 g.) was dissolved in ethanol (120 ml.) and added to a solution of potassium hydroxide (33 g.) in water (33 ml.). The reaction solution

was refluxed for 16 hr. and then cooled. Dilution with water precipitated needles (5.19 g.) which were identified as **1-(4-biphenylyl)cyclopentanecarboxamide (IVd)**, m.p. 149–151° after one crystallization from acetone. An analytical sample melted at 150–151°; λ_{max} 257 mμ (ε 23,400); $\nu_{max}^{CS_2}$ 1692 (amide) cm.$^{-1}$.

Anal. Calcd. for $C_{18}H_{16}NO$: C, 81.47; H, 7.22; N, 5.28. Found: C, 81.80; H, 7.20; N, 5.28, 5.27.

The above filtrate was extracted with ether and acidification of the aqueous solution gave only traces of acidic material. The combined ether extracts were washed with water, dried, and evaporated to dryness leaving a yellow amorphous solid (2.62 g.). This solid was suspended in a 5% aqueous solution (50 ml.) of sodium hydroxide and refluxed for 1 hr. After cooling, the undissolved solid (crude amide) was separated by filtration and the filtrate was acidified with dilute hydrochloric acid. Extraction with ether and working up in the usual way gave **1-(4-biphenylyl)-cyclopentanecarboxylic acid (IVc)** as a white solid (0.21 g.) which melted at 187–189° after one crystallization from aqueous methanol. An analytical sample melted at 200–201°; λ_{max} 256 mμ (ε 24,200); $\nu_{max}^{CS_2}$ 1695 (carboxylic acid) cm.$^{-1}$

Anal. Calcd. for $C_{18}H_{17}O_2$: C, 81.15; H, 6.87. Found: C, 80.79; H, 6.98.

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A Correlation of Drug Concentration with Sterol Biosynthesis Inhibition in the Liver[1]

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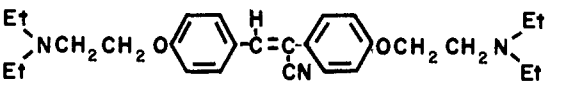
The time course of sterol biosynthesis inhibition of two 2,3-diphenylacrylonitriles which block the conversion of desmosterol to cholesterol is compared with triparanol in orally treated rats. The data thus obtained are correlated with drug concentration in the liver and with the effects of these compounds on sterol biosynthesis *in vitro*.

The liver is regarded as a primary site for the synthesis as well as the metabolism of cholesterol.[2] That a feed-back mechanism is operative in the liver has been shown by cholesterol-feeding experiments which produce higher than normal concentrations of cholesterol in the liver. These livers have a reduced capacity to synthesize new cholesterol.[3,4] Chemical agents also affect liver cholesterol. Estrogens which effectively lower serum cholesterol have been shown to alter both liver sterol content and the ability of these livers to synthesize cholesterol.[5,6] Triparanol, a sterol biosynthesis inhibitor, does not materially change liver sterol concentration, but does alter liver sterol composition. Desmosterol, which is present in minute amounts in normal rat liver, replaces much of the cholesterol in the livers of triparanol-treated animals.[7,8]

In the course of studies of sterol biosynthesis inhibitors[9] that block the conversion of desmosterol to cholesterol, we became interested in correlating drug concentration at the site of action with degree of inhibition. By administering a labeled precursor to animals pretreated with the drug we hoped to determine the extent of biosynthesis inhibition by measuring the relative amounts of labeled desmosterol and cholesterol in the liver and to measure at the same time the drug content of the liver. This report describes an experiment in which the drug content of the liver was correlated with its effect as indicated by the comparison of radioactive liver sterols following mevalonate-2-C^{14} injection. For this purpose two compounds, 2,3-bis[*p*-(2-diethylaminoethoxy)phenyl]acrylonitrile (I) and *trans*-3-[*p*-(2-diethylaminoethoxy)phenyl]-2-phenyl-2-pentenenitrile (II),[9] were compared with triparanol (Chart I).

CHART I

I.



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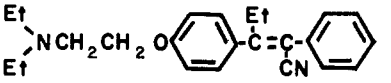
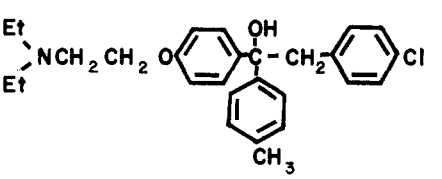


TRIPARANOL



(1) Based on a presentation at the 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., Sept. 10, 1962.

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