[CONTRIBUTION FROM THE STERLING CHEMISTRY LABORATORY AND THE BINGHAM OCEANOGRAPHIC LABORATORY, YALE UNIVERSITY]

Contributions to the Study of Marine Products. XLIX. Synthesis of 29-Isofucosterol[1,2]

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The attempted synthesis of fucosterol by the Wittig reaction has led to an isomer. It has been deduced from its infrared spectrum and the mechanism of its formation that the isomer differs from the natural product in the orientation around the 24,28-double bond, which is *trans* in the former and *cis* in the latter. The new isomer has accordingly been named 29-isofucosterol.

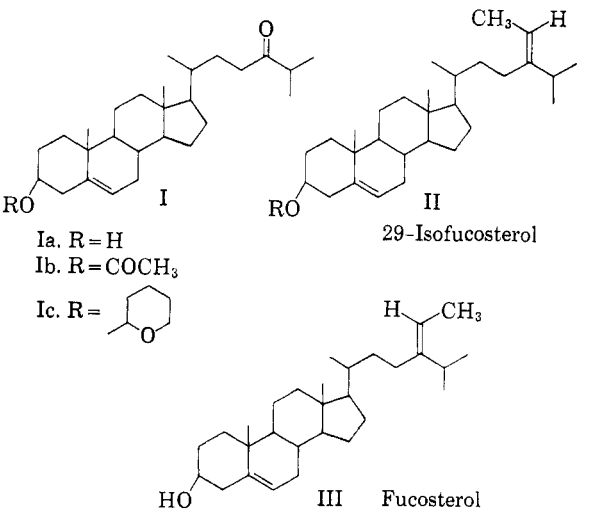
Fucosterol is the typical sterol of the brown algae. Its structure has been established by careful ozonolysis of the sterol to acetaldehyde[4] and 24-ketocholesterol.[5,6] More recently a synthesis of fucosterol has also been reported.[7] Neither the degradative nor synthetic procedures, however, have as yet permitted the assignment of a configuration to the 24,28-double bond of fucosterol.

In connection with studies on the synthesis of natural sterols now in progress in this laboratory, an attempt was made several years ago to prepare fucosterol by means of the Wittig reaction. This reaction has been used with conspicuous success in the synthesis of other sterols.[8] 24-Ketocholesterol (Ia) was converted to its pyranyl ether (Ic) by the previously reported transpyranylation reaction.[9] When this ether was reacted with triphenylphosphonium ethylidene[10] the 24-ethylidene derivative (IIc) was obtained in a rather low yield (20%). Neither changes in reaction times nor temperatures improved the yields, and in all instances more than one half of the starting material was recovered. Removal of the protective group afforded the sterol (IIa) which melted slightly but significantly higher than fucosterol which had been obtained from *Fucus vesiculosus*. (Table I). Even more pronounced was the difference between the melting points of the acetates. Both acetates readily form tetrabromides differing in melting points which revert to the original acetates upon debromination with zinc in acetic acid.

TABLE I

COMPARISON OF FUCOSTEROL AND 29-ISOFUCOSTEROL

	Fucosterol[11]		29-Isofucosterol	
	M.P.	$[\alpha]_D°$	M.P.	$[\alpha]_D°$
Sterol	124	−38.4	128–129	−41.8
Acetate	118–119	−43.8	130.5–131	−41.9
Acetate-tetrabromide	133 (dec.)		138–141 (dec.)	



When ozonized, the new sterol affords acetaldehyde as the volatile fragment, demonstrating the presence of the 24,28-double bond (IIa). The difference between the new sterol and fucosterol must therefore rest in the arrangement of groups around this double bond, and the new sterol may be re-

(1) The material presented in this paper constitutes part of a dissertation submitted by the author in partial fulfillment of the requirements for the Ph.D. degree, Yale University, 1958.

(2) The investigation was in part supported by a research grant, Nonr 253(00), from the Office of Naval Research.

(3) F. W. Heyl Fellow 1957–1958. Present address: Organic Chemical Research Division, American Cyanamid Company, Pearl River, New York.

(4) H. B. MacPhillamy, *J. Am. Chem. Soc.*, **64**, 1732 (1942).

(5) D. H. Hey, J. Honeyman and W. J. Peal, *J. Chem. Soc.*, 2881 (1950).

(6) W. Bergmann and M. Klosty, *J. Am. Chem. Soc.*, **73**, 2935 (1951).

(7) R. Hayatsu, *Pharm. Bull. (Tokyo)*, **5**, 452 (1957). This synthesis involves the reaction of ethylmagnesium bromide with 24-ketocholesterol and the dehydration of the ensuing alcohol with phosphorus oxychloride in pyridine. It is surprising that the dehydration is sufficiently selective to afford fucosterol in a yield of at least 36%.

(8) See for example the recent review by U. Schöllkopf, *Angew. Chem.*, **71**, 260 (1959).

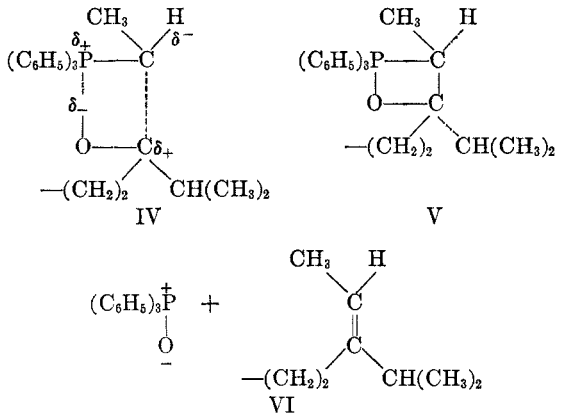
(9) W. Bergmann and J. P. Dusza, *Ann.*, **603**, 36 (1957); *J. Org. Chem.*, **28**, 459 (1958).

(10) G. Wittig and D. Wittenberg, *Ann.*, **606**, 1 (1957); K. Matsiu, *Yûki Gôsei Kagaku Kyôkaishi*, **8**, 211 (1950).

(11) I. M. Heilbron, R. F. Phipers, and H. R. Wright, *Nature*, **133**, 419 (1934); *J. Chem. Soc.*, 1572 (1934).

garded as a 29-isofucosterol. The geometry of the 24,28-double bond of fucosterol appears to be unknown. It has in fact never been mentioned, not even in connection with the recent synthesis.[7,12]

Consideration of the mechanism of the Wittig reaction between 24-ketocholesterol and triphenylphosphonium ethylidene suggests that the *trans*-isomer (IIa) be formed predominantly. The geometry of the intermediates is illustrated by structures IV–VI. Structure IV shows the most favorable arrangement for the transition state in which the methyl group is located *trans* to the isopropyl group which is bulkier in the immediate vicinity than the $(CH_2)_2$-group leading to the ring system. The same geometry should be favored in the four-membered state (V). With the collapse of the intermediate (V), 29-isofucosterol will be formed with the methyl and isopropyl groups in *trans*-arrangement (IIa).



More direct evidence for the assignment of the configurations II and III may be derived from the infrared spectra of the respective sterols. They are virtually identical except for a shift in the wave length of a peak in the 12 micron region (Fig. I). Absorption in this region is associated with unsaturation; it is absent in the tetrabromides. Both sterols show the twin peaks near 11.9μ and 12.5μ attributed to the 5,6-double bond. Each sterol exhibits an additional peak between these two. Absorption in this region may arise from the out-of-plane bending frequency of a hydrogen atom on a trisubstituted ethylene[13] such as the 24,28-double bond of the sterols in question. The peak for this deformation in the synthetic material occurs at longer wavelength (12.30μ) than in the natural product (12.14μ). One would expect the *cis*-arrangement of groups (III) to give maximum interference between the methyl and the isopropyl groups, to impair the bending mode of the hydrogen

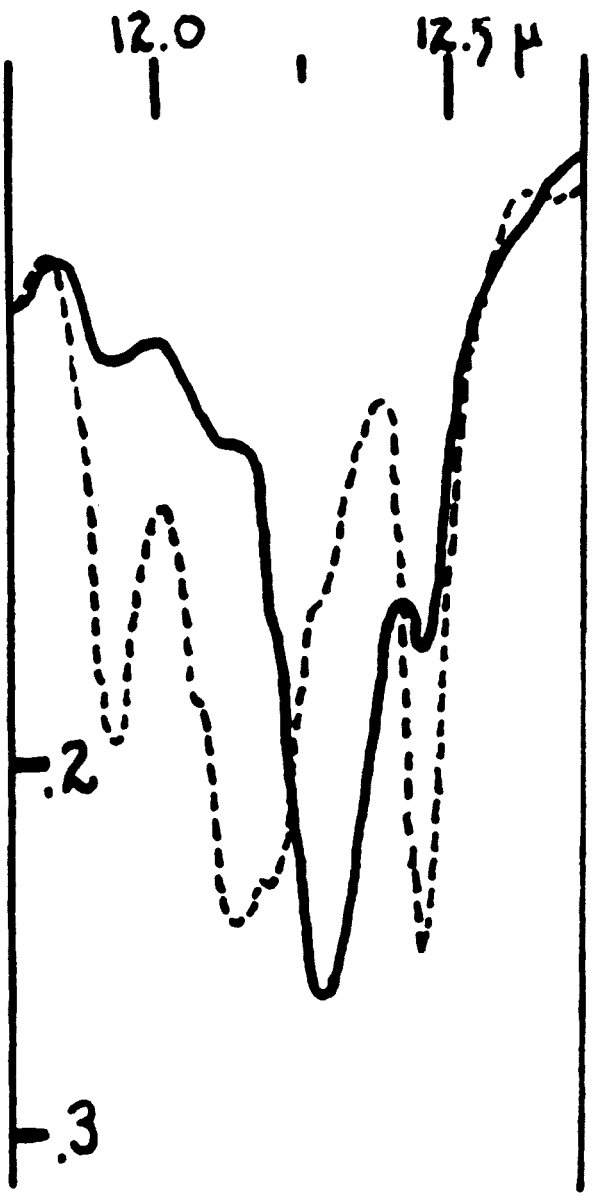


Fig. 1. Infrared spectra in KBr of the acetates of 29-isofucosterol (———) and fucosterol (– – – –) in the 12-micron region

atom at C-28 and to move the band to shorter wavelength. On the basis of such considerations structure III should be assigned to the natural product, fucosterol, and structure II to 29-isofucosterol.

These considerations derive support from the infrared spectra of pairs of simple olefins which have been published in the American Petroleum Institute Series. Here the *cis*-isomers show the deformation in question at the lower wave lengths. This is best illustrated by the 3,4-dimethyl-2-pentenes which are analogous to the sidechains of fucosterol (III) and 29-isofucosterol (II). The *cis*-isomer (VIII) exhibits the peak at 12.18μ,[14] and

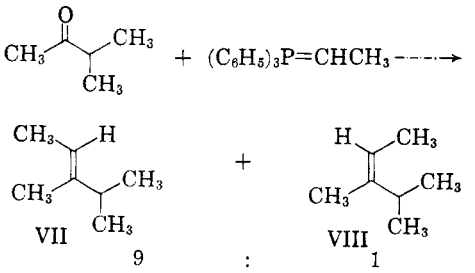
(12) More recently, however, attention has been called to the problem by Y. Mazur and F. Sondheimer, *J. Am. Chem. Soc.*, **80**, 6296 (1958) in connection with the structure of citrostadienol which also carries a 24,28-double bond.

(13) L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, John Wiley and Sons, New York, 1954, p. 51–52.

(14) Infrared Spectral Data, American Petroleum Institute Research Project 44, Serial Number 1796.

the *trans*-isomer (VII) at 12.39μ.[15] As the analogous peak is at the lower wavelength in the fucosterol spectrum, 12.14μ with a shoulder at 12.18μ, we may conclude that the natural product is the *cis*-isomer (III), and that the synthetic 29-isofucosterol with a peak at 12.30μ is the *trans*-isomer (II).

For comparative studies, the Wittig reaction between methyl isopropyl ketone and triphenylphosphonium ethylidene was also investigated. The olefin obtained was a mixture of 3,4-dimethyl-2-pentenes, the infrared spectrum of which showed strong peak at 12.33μ with a shoulder at 12.18μ. Gas chromatography of the mixture indicated an approximate 9:1 ratio of *trans*- (VII) to *cis*-isomer-(VIII).



EXPERIMENTAL

All melting points were taken in open capillary tubes in a Hershberg apparatus equipped with Anschütz thermometers. Optical rotations were determined with a polarimeter having a Rudolph photoelectric attachment on samples in a 1-dm. tube. Samples were dissolved in chloroform with the concentration given in each case. The infrared spectra were determined on a Perkin-Elmer Model 21 Recording Spectrophotometer as potassium bromide pellets or as otherwise stated. All values were corrected against an atmosphere spectrum.

24-Ketocholesteryl-3β-(2′-tetrahydropyranyl)-ether (Ic). To a suspension of 6.00 g. of 24-ketocholesterol in 50 ml. of 2-methoxytetrahydropyran[16] was added 2.0 g. of Dowex-50 (dried at 70° for 24 hr.). This was stirred and kept at 90° for 7 hr. under a slow stream of nitrogen, the flask being capped with a calcium chloride tube. After cooling the resin was filtered and the excess solvent removed *in vacuo*. The residue was taken up in hexane and passed through a neutral alumina column. The eluate was evaporated to dryness and crystallized from acetone to give 5.70 g. of pyranyl ether, m.p. 115–121°. An additional 0.35 g. could be obtained on slight concentration of the mother liquor. The analytical sample melted at 131–132°, $[\alpha]_D^{26}$ −30.4° (*c*, 1.18); λ_{max} 5.86 and 8.99 μ.

Anal. Calcd. for $C_{32}H_{50}O_3$: C, 79.28; H, 10.81. Found: C, 79.10; H, 10.57.

No attempt was made to separate the optical isomers formed on preparation of this derivative.

29-Isofucosteryl-3β-(2′-tetrahydropyranyl)-ether (IIc). A suspension of 1.95 g. of triphenylethylphosphonium bromide[10] in 25 ml. of absolute ether (distilled from phenylmagnesium bromide) was stirred in a pressure flask (175 ml. capacity). To this was added 4.0 ml. of 1.3*N* butyllithium solution[17] with the characteristic development of the clear red-orange solution. After this solution had been stirred for 1 hr., 2.5 g. of 24-ketocholesteryl-3β-(2′-tetrahydropyranyl)-ether dissolved in 35 ml. of absolute ether was added followed by an additional 24 ml. of absolute ether which promoted the stirring of the precipitated material.

An additional hour of stirring at room temperature was provided and then the flask was sealed and placed in an oil bath which was heated to 65° and held there for 16 hr. After cooling, the excess reagent was decomposed with the addition of water and the mixture filtered. The ether was evaporated to dryness.

The residues from two identical reactions were combined and taken up in hexane. This was chromatographed on a Silicic acid-Celite 545 column (150 g.:75 g.). The pyranyl ether was eluted with benzene-hexane (3:1) and with benzene to give 1.02 g. of the ether, which was usually depyranylated directly without further purification. In one preparation the pyranyl ether was crystallized from ethanol to give a gel which slowly crystallized, m.p. 105–107°; $[\alpha]_D^{28}$ −18.9° (*c*, 0.90).

Anal. Calcd. for $C_{34}H_{56}O_2$: C, 82.20; H, 11.36. Found: C, 82.05; H, 11.25.

The column was stripped with ether. Upon evaporation of the solvent, 2.45 g. of starting material was recovered.

29-Isofucosterol (IIa). A solution of 1.02 g. of 29-isofucosteryl-3β-(2′-tetrahydropyranyl)-ether in 50 ml. of hexane was added to a solution of four drops of concentrated hydrochloric acid in 50 ml. of methanol and the two-phased system was allowed to stand at room temperature for 1 hr. with intermittent stirring. The solvents were removed *in vacuo* and the residue crystallized from methanol to give 0.79 g. of the sterol, m.p. 120–122°. After several crystallizations an analytical sample showed the following physical constants, m.p. 128–129°; $[\alpha]_D^{26}$ −41.8°[18] (*c*, 1.20); λ_{max}^{KBr} 11.91, 12.30 and 12.50 μ; $\lambda_{max}^{CS_2}$ 11.93, 12.27 and 12.50 μ.

Anal. Calcd. for $C_{29}H_{48}O$: C, 84.40; H, 11.72. Found: C, 84.74; H, 11.81.

29-Isofucosteryl acetate (IIb). A solution of 610 mg. of the sterol in 15 ml. of acetic anhydride and 15 ml. of dry pyridine was kept overnight at room temperature. The acetylation mixture was poured into water and the crude acetate filtered. On crystallization from methanol there was obtained 0.55 g. of the acetate, m.p. 122–125°. After several more crystallizations and drying *in vacuo*, the acetate melted at 130.5–131°, $[\alpha]_D^{27}$ −41.9° (*c*, 1.51); λ_{max}^{KBr} 5.77, 8.02, 11.92, 12.30 and 12.47 μ; $\lambda_{max}^{CS_2}$ 5.76, 8.10, 11.92, 12.30 and 12.48 μ.

Anal. Calcd. for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found: C, 82.23; H, 11.21.

29-Isofucosteryl acetate 5,6,24,28-tetrabromide. A sample of 29-isofucosteryl acetate, 110 mg., was dissolved in 1.0 ml. of absolute ether. To this solution was added 2.0 ml. of a 5% solution of bromine in glacial acetic acid (wt./wt.). A precipitate began forming immediately. The solution was refrigerated overnight and then filtered. After washing with acetic acid and methanol, there was obtained 80 mg. of the tetrabromide, m.p. 139–141° dec.; λ_{max} 5.75 and 8.09 μ. No absorption between 12.0 and 12.5 μ.

Anal. Calcd. for $C_{31}H_{50}O_2Br_4$: C, 48.08; H, 6.53. Found: C, 48.38; H, 6.50.

Debromination of 29-isofucosteryl acetate tetrabromide. The tetrabromide was suspended in 1.5 ml. of dry ether and 10

(15) Infrared Spectral Data, American Petroleum Institute Research Project 44, Serial Number 1904.

(16) R. Paul, *Bull. soc. chim. France*, [5], **1**, 973 (1934); G. F. Woods and D. N. Kramer, *J. Am. Chem. Soc.*, **69**, 2246 (1947).

(17) H. Gilman and A. H. Haubein, *J. Am. Chem. Soc.*, **66**, 1515 (1944).

(18) The molecular rotation contribution of the 24-ethylidene group in 29-isofucosterol[MD(29-isofucosterol)-MD(cholesterol)] is −22, or of the same magnitude and direction as the corresponding contribution of the 24-ethylidene group in fucosterol, −18 (D. H. R. Barton, *J. Chem. Soc.*, 813 (1945)). In contrast the contribution of the 24-ethylidene group of unknown configuration in citrostadienol has been reported as +90; see ref. 12.

drops of glacial acetic acid and 50 mg. of zinc dust was added. After 1 min. of stirring the tetrabromide dissolved. Occasional stirring was provided during an additional 15 min. period. Ether was added to the solution and this was decanted from the zinc. The ether solution was washed with water and dried. Crystallization of the residue left after evaporation of the ether gave 30 mg. of the acetate, m.p. 129–131°. An additional crystallization sharpened the melting point to 130.5–131°. The infrared spectrum of this material was identical in every minute detail to that of 29-isofucosteryl acetate. No change had occurred in the structure of the steryl acetate during the bromination and debromination steps.

Ozonolysis of 29-isofucosteryl acetate. A solution of 260 mg. of the acetate in 50 ml. of cold purified glacial acetic acid was subjected to a stream of ozone (6%) for 5 min. Zinc dust was added to decompose the ozonide. This was shaken and then filtered. The water from a gas scrubber (25 ml.) used in the ozonolysis train was added to the acetic acid solution and the material was distilled until approximately 25 ml. of distillate was collected.

The distillate was poured into a solution of 2,4-dinitrophenylhydrazine in 2*N* hydrochloric acid. The derivative precipitated immediately. After filtration, the material was dissolved in chloroform and chromatographed on a column of Bentonite:Celite 545 (3:1).[19] The derivative was eluted with chloroform-ethanol (10:1). Crystallization from ethanol gave the 2,4-dinitrophenylhydrazone of acetaldehyde, m.p. 160–162°. This was identical to an authentic sample (mixed melting point, infrared spectrum, paper chromatogram).

Isolation of fucosterol from Fucus vesciculosus. The ether extract of the air-dried material was saponified to give the pure sterol after several crystallizations from methanol, m.p. 122–123°; λ_{max} 11.90, 12.17 and 12.50 μ. The sterol was acetylated to give the acetate. Crystallization from methanol gave the pure acetate, m.p. 119–120°; λ_{max} 5.77, 6.00, 8.02, 11.92, 12.14, 12.19 (sh) and 12.47 μ. Addition of bromine gave the acetate tetrabromide, m.p. 131–133° dec. Debromination with zinc and acetic acid gave back fucosteryl acetate.

Wittig reaction of methyl isopropyl ketone with triphenylphosphonium ethylidene. To a heavy slurry of 13.15 g. (35.4 mmol.) of triphenylethylphosphonium bromide in 15 ml. of absolute ether was added 25.9 ml. of 1.3*N* butyllithium solution (33.7 mmol.). The solution was stirred for 1 hr. to give a red solution with the suspended excess salt. The solution was cooled to 0° and 3.62 ml. of dry redistilled methyl isopropyl ketone (2.90 g., 33.7 mmol.) was added and the heavy precipitate stirred for 1 hr. The pressure flask was then capped and heated to 65° for 3 hr. in an oil bath.

After cooling, the flask was opened and a column attached and as much ether distilled (bath 70°) as possible. The pressure was reduced and the distillate collected in a Dry Ice-cooled receiver. This material was distilled and gave 0.45 g. of olefin with a considerable loss due to hold-up. The following physical constants were obtained after another vacuum distillation; b.p. 85–88° (microdetermination); n_D^{28} 1.4163; λ_{max}^{neat} 6.00, 7.25, 7.30, 7.37, 12.18–12.27 (sh) and 12.33μ; reported for 3,4-dimethyl-2-pentene (*cis* or *trans* not specified) b.p. 86.2–86.4°, n_D^{25} 1.4052[20]; b.p. 85–89°, n_D^{27} 1.4100[21]; b.p. 91°, n_D^{21} 1.4135[22]; b.p. 87°, n_D^{21} 1.404.[23]

Gas chromatography of a sample of the olefin in a four meter column and operated at 85° showed an isomer ratio of 9:1.

Acknowledgment. The author is indebted to Professor Werner Bergmann for his advice, encouragement and suggestions during the course of this work.

NEW HAVEN, CONN.

(19) J. W. White, *Anal. Chem.*, **20**, 725 (1948); J. A. Elvidge and M. Whalley, *Chem. and Ind.* (London), 589 (1955).

(20) F. J. Soday and C. E. Boord, *J. Am. Chem. Soc.*, **55**, 3293 (1933).

(21) I. N. Narsarov, *Ber.*, **70**, 617 (1937).

(22) A. Guillemonat, *Ann. Chim.*, **11**, 143 (1939).

(23) Selective Values of Properties of Hydrocarbons, National Bureau of Standards, Circular 0461, November 1947, Washington, p. 49.

[CONTRIBUTION FROM THE DIVISION OF CHEMICAL RESEARCH OF G. D. SEARLE AND CO.]

Steroidal Aldosterone Blockers. III[1,2]

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The syntheses and biological activities of several new steroidal 17-spirolactones are presented. Oxygenation of position 11 in the steroid nucleus produces some increase of aldosterone blocking activity and this activity is further enhanced by the additional introduction of 9-α-fluoro substituent.

Earlier articles[1] in this series reported on a number of steroidal 17-spirolactones bearing modifications in the lactone and 3-oxo-4-ene systems; nuclear unsaturation and acylthio substituents were also introduced. This article reports on a number of oxygenated steroidal 17-spirolactones and related derivatives. The basic structures subjected to modification in this work were those of 3-(3-oxo-17β-hydroxy-4-androsten-17-α-yl)propanoic acid lactone (Ia) and its 19-nor analog (Ib).

Monohydroxy derivatives of Ia and Ib were prepared both by adrenal perfusion[3] and by fermentation with a species of *Rhizopus*. Perfusion has been shown to give predominantly 11β-hydroxylation.[4] The products of perfusion, IIe and IIb, were oxi-

(1) Paper II, J. A. Cella and R. C. Tweit, *J. Org. Chem.*, **24**, 1109 (1959).

(2) Presented in part before the Division of Medicinal Chemistry at the 135th National Meeting of the American Chemical Society, Boston, Mass., April 1959.

(3) These perfusions were carried out at the Worcester Foundation for Experimental Biology, Shrewsbury, Mass., by Mr. Austin Fish.