N HCl. The mixture was refluxed for 1 hr., then diluted with 100 ml. of water, cooled in an ice bath, and made slightly alkaline with 0.1 *N* NaOH solution. Filter aid (3 g.) was stirred into the mixture and left for 0.5 hr. The precipitate was collected and washed well with water. The filter cake was then extracted with five 40-ml. portions of boiling ethanol. The alcohol was removed *in vacuo* and the yellow residue was recrystallized from aqueous ethanol; yield, 0.62 g. (93%); m.p. above 300° (dec.); λ_{max} 2.95–3.60 (broad OH, NH, CH), 12.30 (*p*-C_6H_4), 12.80 μ (C–Cl). See Table III for other data.

2-{N-[1-(2-Amino-4-hydroxy-6-methyl-5-pyrimidyl)-3-propyl]-4-aminophenyl}ethyl Chloromethyl Ketone (VIIIb).—A solution of 350 mg. (0.86 mmole) of XVIb in 25 ml. of 0.1 *N* aqueous HCl was heated on a steam bath for 1 hr. The pH of the solution was then adjusted to 9 with 0.1 *N* aqueous NaOH. The product was collected on a filter, washed with 10 ml. of water, recrystallized from ethanol–water, and gave light yellow crystals; yield, 200 mg. (64%); m.p. >300°; λ_{max} 2.90–3.40 (broad OH, NH, CH), 5.79 (C=O), no C–O–C band near 9.6 μ; $\lambda_{max}^{pH\ 13}$ 240 mμ (ϵ 12,700), 279 mμ (ϵ 6800); $\lambda_{max}^{pH\ 1}$ 217 mμ (ϵ 12,800), 265 mμ (ϵ 7100).

Other compounds prepared by a similar hydrolysis are listed in Table III under method F.

2-Amino-5-(*p*-*n*-butylanilinopropyl)-6-methyl-4-pyrimidinol (XVIII).—A solution of 1.49 g. (10 mmoles) of *p*-*n*-butylaniline[16] and 2.23 g. (10 mmoles) of I in 100 ml. of methanol was allowed to react for 30 min. With magnetic stirring 2 g. of sodium borohydride was added in portions over a period of 30 min. After being stirred overnight, 20 ml. of 5% aqueous NaOH was added and the methanol was removed *in vacuo*. The solution was diluted with 100 ml. of water and the pH was adjusted to 9 with 5% HCl. The precipitated product was collected and recrystallized from methanol–water; yield, 2.3 g. (73%); m.p. 223–225°; λ_{max} 2.90–3.60 (broad OH, NH, CH), 6.17, 6.58 (NH, C=N, C=C), 12.38 μ (C_6H_5–); $\lambda_{max}^{pH\ 1}$ 265 mμ (ϵ 9300); $\lambda_{max}^{pH\ 7}$ 240 mμ (ϵ 15,600), 291 mμ (ϵ 6300); $\lambda_{max}^{pH\ 13}$ 240 mμ (ϵ 17,200), 280 mμ (ϵ 7900).

Anal. Calcd. for $C_{18}H_{26}N_4O$: C, 68.8; H, 8.33; N, 17.8. Found: C, 68.5; H, 8.50; N, 17.7.

(16) R. R. Read and D. B. Mullin, *J. Am. Chem. Soc.*, **50**, 1763 (1928).

Synthesis of Fatty Acids with Smooth Muscle Stimulant Activity

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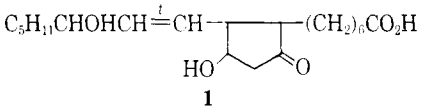
12-Hydroxyheptadec-*trans*-10-enoic acid has been synthesized by reduction of the corresponding acetylenic derivative with lithium in liquid ammonia at room temperature after pretreatment with lithium hydride to avoid hydrogenolysis. This acid, which represents a fragment of a prostaglandin, has been found to be three times as active as ricinelaidic acid in stimulating the isolated hamster colon. Homologs and other derivatives, including some containing an additional 6-oxo or 6-hydroxy group, have been synthesized and their activity has been determined. The hydroxyacetylenic acids were synthesized *via* chloroalkynols from alk-1-yn-3-ols and were converted into hydroxyoxoacetylenic acids *via* reaction of the acid chlorides with cycloalkenamines.

Interest has recently been renewed in the pharmacological potentialities of fatty acids largely because of the elucidation of the nature of the prostaglandins.[2] Consideration of the structure of prostaglandin E_1 (**1**) and of the pharmacologically active acids, notably ricinelaidic acid (**2**), among those examined for smooth muscle stimulant activity,[3,4] suggests that common features may include a hydroxyl group at position 12 and unsaturation or structural rigidity between positions 8 and 11 in an unbranched aliphatic acid.



1

$C_6H_{13}CHOHCH_2CH\overset{t}{=}CH(CH_2)_7CO_2H$

2

$RCHOHC{\equiv}C(CH_2)_nCO_2H$

3

Synthesis of acetylenic acids of type **3** was therefore investigated. One previous example (**3**, R = C_6H_{13}; n = 6) has been reported,[5] synthesized in unstated yield by a route involving the reaction of heptanal with lithium 8-chlorooctyne. From work with dimethylundecynamide (described below) there was reason to believe that higher alkynes might be unreactive so the following route was examined. This was

$$RCHO \longrightarrow \underset{\mathbf{4}}{RCHOHC{\equiv}CH} \longrightarrow \underset{\mathbf{5}}{RCHOPyC{\equiv}CH} \longrightarrow$$

$$\underset{\mathbf{6}}{RCHOHC{\equiv}C(CH_2)_nCl} \longrightarrow \underset{\mathbf{7}}{RCHOHC{\equiv}C(CH_2)_nCN} \longrightarrow$$

$$\underset{\mathbf{8}}{RCHOHC{\equiv}C(CH_2)_nCO_2Me} \longrightarrow$$

$$RCHOHCH\overset{c}{=}CH(CH_2)_nCO_2Me$$

9

Py = 2-tetrahydropyranyl

tested using the readily available hexynol (**4**, R = C_3H_7) to give the ester (**8**, R = C_3H_7; n = 6). Difficulties arose when the route was extended to use oct-1-yn-3-ol (**4**, R = C_5H_{11}). This had been previously prepared[6] by selenium dioxide oxidation of 1-octyne. This method is unsuitable for large-scale preparation so the familiar reaction of sodium acetylide with an aldehyde was used. However, it was found that with hexanal, particularly commercial samples, the yield and purity of octynol was very variable and frequently only higher boiling unsaturated hydroxy ketones were obtained. Similar difficulties were found with heptanal,[7] or when ethynylmagnesium bromide was used.

(1) School of Pharmacy, College of Technology, Park Road, Portsmouth, Hampshire, England.

(2) S. Bergström, R. Ryhage, B. Samuelsson, and J. Sjövall, *Acta Chem. Scand.*, **16**, 501 (1962).

(3) N. Ambache, *J. Physiol.* (London), **146**, 255 (1959); N. Ambache, M. Reynolds, and J. M. C. Whiting, *ibid.*, **166**, 251 (1963).

(4) M. S. Masri, L. A. Goldblatt, F. DeEds, and G. O. Kohler, *J. Pharm. Sci.*, **51**, 999 (1962).

(5) A. S. Bailey, V. G. Kendall, P. B. Lumb, J. C. Smith, and C. H. Walker, *J. Chem. Soc.*, 3027 (1957).

(6) R. Truchet, *Compt. rend.*, **196**, 708 (1933).

(7) L. Crombie, personal communication, 1963.

The best yield was obtained with freshly prepared hexanal–sodium bisulfite and sodium acetylide in liquid ammonia.[8] The acetylenic ester (**8**, R = C_5H_{11}; n = 8) was then prepared and reduced catalytically to the *cis* ester (**9**, R = C_5H_{11}; n = 8). Attempted isomerization of this with selenium at 200° caused dehydration and the acetyl derivative was thermally unstable.[9] Direct preparation of *trans* derivatives was first tried on the chlorides (**6**), but both lithium aluminum hydride and lithium–ammonia caused extensive loss of chlorine. Salts of the acids (**3**) were too insoluble for effective reduction in liquid ammonia at −33°, and at room temperature[10] the hydroxyl group was hydrogenolyzed. This was prevented by pretreatment with lithium hydride, and the *trans* acid (**10**, R = C_5H_{11}; n = 8) was prepared. It was found to have a strong absorption band at 970 cm.$^{-1}$ absent in the *cis* isomer.

$$\underset{\mathbf{10}}{\text{RCHOHCH}\overset{t}{=}\text{CH(CH}_2)_n\text{CO}_2\text{H}}$$

In an attempt to estimate the purity of the geometrical isomers, their separation by thin layer chromatography was examined. Methyl oleate is separated from methyl elaidate on plates incorporating silver nitrate,[11] but the methyl ethers[12] of methyl ricinoleate and ricinelaidate are separated only on freshly prepared plates.[13] Derivatives of the isomers of the new acids were not separated in this way, and plates incorporating cuprous, mercurous, and palladous salts were also ineffective.

In order to determine the effect on biological activity of increasing the separation between carboxyl and hydroxyl groups, the esters **8** (R = C_5H_{11}; n = 9, and R = C_4H_9; n = 10) were prepared. A trihydroxy acid was prepared from **9** (R = C_5H_{11}; n = 9).

An alternative approach to the acetylenic acids was tried using N,N-dimethylundec-10-ynamide.[14] However the lithium derivative in liquid ammonia would not react with butanal or hexanal, and suitable silver or mercury derivatives could not be obtained. Zerewitinoff determinations showed the ethynyl hydrogen to be unreactive with alkylmagnesium halide at room temperature.

Because the prostaglandins bear a hydroxyl group β to the double bond, the preparation of acids of type **11** was investigated. 1-Chloro-9,10-epoxyundecane

$$\underset{\mathbf{11}}{\text{RCHOHC}{\equiv}\text{CCH}_2\text{CHOH(CH}_2)_n\text{CO}_2\text{H}}$$

was prepared but failed to react with hexynyllithium. The sodio derivative of dimethyl 3-oxo-undecanedioate reacted with 1-iodo-oct-3-yne, but only unsubstituted diester was recovered. Methyl sebacaldehydate reacted with propargylaluminum bromide to give[15] methyl 10-hydroxytridec-12-ynoate, but with 1-bromo-oct-2-yne a complex mixture including allenic products was formed. Attempts to prepare a silver derivative of the tridecynoate were unsuccessful.

Prostaglandins of the E series bear a keto group δ to the double bond, and those of the F series have a second hydroxyl group in this position. Preparation of esters of types **16** and **17** was therefore undertaken by the route shown.

$$\underset{\mathbf{12}}{\text{RCHOHC}{\equiv}\text{C(CH}_2)_3\text{CO}_2\text{H}} \longrightarrow \underset{\mathbf{13}}{\text{RCHOAcC}{\equiv}\text{C(CH}_2)_3\text{CO}_2\text{H}} \longrightarrow \underset{\mathbf{14}}{\text{RCHOAcC}{\equiv}\text{C(CH}_2)_3\text{COCl}} \longrightarrow \underset{\mathbf{15}}{\text{RCHOAcC}{\equiv}\text{C(CH}_2)_3\text{COCH}\!-\!\text{CO}\ (\text{CH}_2)_{n-1}} \longrightarrow \underset{\mathbf{16}}{\text{RCHOHC}{\equiv}\text{C(CH}_2)_3\text{CO(CH}_2)_n\text{CO}_2\text{Me}} \longrightarrow \underset{\mathbf{17}}{\text{RCHOHC}{\equiv}\text{C(CH}_2)_3\text{CHOH(CH}_2)_n\text{CO}_2\text{Me}}$$

The acyl chlorides (**14**) reacted with morpholinocycloalkenes to give, after hydrolysis, the diketones (**15**) which were cleaved to the keto acids (**16**). The route was tested by preparing compounds in which R = C_3H_7; n = 5 and then used to prepare the esters **16** and **17** (R = C_5H_{11}; n = 4). Corresponding *cis* esters were prepared by catalytic reduction.

Finally some approaches were made to the synthesis of acids of type **18**, containing all the functional groups of prostaglandin E_1, *via* acids of type **19**. 1-Chloro-

$$\underset{\mathbf{18}}{\text{RCHOHCH}\overset{t}{=}\text{CHCH}_2\text{CHOHCH}_2\text{CO(CH}_2)_n\text{CO}_2\text{H}}$$

$$\underset{\mathbf{19}}{\text{RCHOHCH}\overset{t}{=}\text{CHCH}_2\text{CHOHCH}_2\text{CO}_2\text{H}}$$

2,3-epoxypropane reacted with hexynylmagnesium bromide to give only 3-bromo-1-chloropropan-2-ol. It has been reported[16] to form rearranged products with sodium alkynyls. 3-Iodo-1-chloropropan-2-ol[17] was converted to a pyranyl derivative, but this failed to react with hexynylmagnesium bromide, and reacted by elimination of hydrogen iodide with lithium hexynyl. Non-1-en-4-yn-6-ol was prepared, but selective epoxidation could not be achieved and attempted hypohalite addition caused decomposition. 1,1-Diethoxyoct-*trans*-3-en-5-ol was prepared, but conditions suitable for preparation of the aldehyde caused much rearrangement of the double bond. Allylic bromination of derivatives of non-*trans*-2-en-4-ol gave a mixture of unstable products.

12-Hydroxyheptadec-*trans*-10-enoic acid, dissolved in aqueous sodium bicarbonate, was found to have a stimulant activity on the isolated hamster colon of about three times that of ricinelaidic acid, the most active of the aliphatic acids tested hitherto.[3] In this preparation prostaglandin E_1 has an activity of about 100 times that of ricinelaidic acid. Samples of other acids were prepared from pure esters and were similarly tested without further purification. 12-Hydroxy-*cis*-10-enoic and 12-hydroxy-10-ynoic acids were about as active as ricinoleic acid. Introduction of a 6-oxo group did not affect activity but introduction of a 6-hydroxy group caused it to fall to about one-tenth.

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(9) *Cf.* L. J. Morris, R. T. Holman, and K. Fontell, *J. Lipid Res.*, **1**, 412 (1960).
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(15) E. Truscheit and K. Eiter, *Ann. Chem.*, **658**, 66 (1962).
(16) L. J. Haynes, I. Heilbron, E. R. H. Jones, and F. Sondheimer *J. Chem. Soc.*, 1583 (1947).
(17) M. V. Grignard, *Bull. soc. chim. France*, **29**, 944 (1903).

TABLE I

HYDROXY UNSATURATED ESTERS AND PRECURSORS

R	X	n	Y	B.p., °C.	mm.	Formula	% calcd. C	% calcd. H	% calcd. Cl	% found C	% found H	% found Cl
C_3H_7	C≡C	3	Cl	80	0.25	$C_9H_{15}ClO$	62.1	8.4	20.3	62.5	8.6	20.3
C_3H_7	C≡C	6	Cl	120–121	0.8	$C_{12}H_{21}ClO$	66.6	9.7	16.3	66.2	9.7	16.4
C_4H_9	C≡C	10	Cl	155–156	0.45	$C_{17}H_{31}ClO$	71.4	10.9	12.4	70.2	10.8	13.5
C_5H_{11}	C≡C	3	Cl	111	0.5	$C_{11}H_{19}ClO$	65.2	9.5	17.5	65.1	9.3	17.6
C_5H_{11}	C≡C	8	Cl	135–137	0.2	$C_{16}H_{29}ClO$	70.5	10.7	13.0	69.7	11.1	13.3
C_3H_7	C≡C	3	CO_2Me	94–95	0.2	$C_{11}H_{18}O_3$	66.6	9.2		66.9	9.4	
C_3H_7	C≡C	6	CO_2Me	121–122	0.3	$C_{14}H_{24}O_3$	70.0	10.0		70.1	10.1	
C_4H_9	C≡C	10	CO_2Me	158	0.1	$C_{19}H_{34}O_3$	73.5	11.0		73.7	11.0	
C_5H_{11}	C≡C	3	CO_2Me	130	0.8	$C_{13}H_{22}O_3$	69.0	9.8		69.4	9.9	
C_5H_{11}	C≡C	8	CO_2Me	151	0.35	$C_{18}H_{32}O_3$	72.9	10.9		73.1	11.0	
C_5H_{11}	C≡C	9	CO_2Me	164–166	0.35	$C_{19}H_{34}O_3$	73.5	11.0		73.8	11.3	
C_3H_7	CH=CH (*c*)	3	CO_2Me	80–82	0.1	$C_{11}H_{20}O_3$	66.0	10.0		65.8	10.1	
C_5H_{11}	CH=CH (*c*)	8	CO_2Me	158	0.5	$C_{18}H_{34}O_3$	72.4	11.5		72.3	11.6	
C_5H_{11}	CH=CH (*c*)	9	CO_2Me	152	0.2	$C_{19}H_{36}O_3$	73.0	11.6		73.6	11.5	
C_3H_7	CH=CH (*t*)	3	CO_2Me	83–84	0.1	$C_{11}H_{20}O_3$	66.0	10.0		65.6	10.2	
C_5H_{11}	CH=CH (*t*)	3	CO_2Me	124–126	0.6	$C_{13}H_{24}O_3$	68.4	10.6		68.8	10.7	
C_5H_{11}	CH=CH (*t*)	8	CO_2Me	142–144	0.15	$C_{18}H_{34}O_3$	72.4	11.5		73.0	12.0	

Increase of the polymethylene chain from 8 to 9 reduced the activity to below one-hundredth.

Experimental[18]

Oct-1-yn-3-ol. A.—Sodium (about 0.5 g.) was dissolved in liquid ammonia (1 l.) and a few crystals of ferric nitrate were added. The blue solution turned to a gray suspension of sodamide. Sodium (20 g.) was added in pieces over 2 hr. Acetylene was passed through the suspension at 1 l. min.$^{-1}$ for 3 hr., the flow rate was then halved, and dry redistilled hexanal (50 g., 0.5 mole) in dry ether (100 ml.) was added over 1.5 hr. The mixture was left for 16 hr. Ammonium chloride (50 g.) was added, the ammonia was allowed to evaporate, and the residue was treated with saturated NH_4Cl and ether. The organic layer was dried (Na_2SO_4), concentrated, and distilled to give 26.5 g. (42%) of an oil, b.p. 44–47° (1 mm.).

B.—A similar procedure using sodium (46 g.) and hexanal-bisulfite[19] (258 g.) gave on distillation 104 g. (66%) of an oil, b.p. 87–89° (21 mm.); $n^{25}D$ 1.4398; ν_{max} 3400, 3300, 2120 cm.$^{-1}$.

Anal. Calcd. for $C_8H_{14}O$: C, 76.1; H, 11.2. Found: C, 75.8; H, 11.1.

C.—Ethynylmagnesium bromide was prepared[20] from bromoethane (176 g.) in dry tetrahydrofuran (1680 ml.), cooled in ice, and dry redistilled hexanal (154 g.) was added dropwise. The mixture was left at room temperature for 18 hr., decomposed with saturated NH_4Cl, and extracted with ether. This was dried and concentrated to give an oil (180 g.) which was distilled to give 79 g. (40%) of an oil, b.p. 75–85° (10 mm.).

2-(Hex-1-yn-3-yloxy)tetrahydropyran (4, R = C_3H_7).—Hex-1-yn-3-ol (22 ml., 0.2 mole) and dihydropyran (18 ml., 0.2 mole) were mixed and concentrated HCl (4 drops) was added. The mixture was allowed to become warm and was then kept for 16 hr. It was then rapidly diluted with ether, washed with saturated $NaHCO_3$, dried (Na_2SO_4), concentrated, and distilled to give 30.3 g. (84%) of an oil, b.p. 88–92° (8 mm.), redistilled b.p. 95° (10 mm.); ν_{max} 3300, 2120, 970 cm.$^{-1}$.

Anal. Calcd. for $C_{11}H_{18}O_2$: C, 72.5; H, 10.0. Found: C, 72.7; H, 9.9.

Similarly prepared was 2-(hept-1-yn-3-yloxy)tetrahydropyran (4, R = C_4H_9), b.p. 110–112° (10 mm.).

Anal. Calcd. for $C_{12}H_{20}O_2$: C, 73.4; H, 10.3. Found: C, 73.4; H, 10.2.

2-(Oct-1-yn-3-yloxy)tetrahydropyran (4, R = C_5H_{11}), b.p. 74° (0.5 mm.), was also prepared.

Anal. Calcd. for $C_{13}H_{22}O_2$: C, 74.2; H, 10.5. Found: C, 74.3; H, 10.5.

Chloroalkynols (6).—In dry distilled liquid ammonia (500 ml.), lithium (1.55 g., 0.22 g.-atom) was converted to lithium amide, and the alkynyloxytetrahydropyran (0.2 mole) added over 45 min. The black suspension was stirred for 45 min. and α-chloro-ω-iodoalkane or α-chloro-ω-bromoalkane (0.21 mole) added over 15 min. The mixture was stirred for 3 hr., the ammonia was allowed to evaporate overnight, and the residue was treated with water and extracted with ether. This was dried (Na_2SO_4) and concentrated to give an oil which was dissolved in methanol (500 ml.), water (50 ml.), and concentrated HCl (50 ml.). The solution was kept at reflux for 3 hr., cooled, diluted, and extracted with ether. This was washed with saturated $NaHCO_3$, dried (Na_2SO_4), concentrated, and distilled; yield 70–80%; ν_{max} 3400, 2230 cm.$^{-1}$. In this way the chlorides in Table I were prepared.

Hydroxyalkynonitriles (7).—A solution of the chloroalkynol (0.19 mole) and dry sodium iodide (30 g., 0.2 mole) in dry acetone (250 ml.) was kept at reflux for 5 hr. and then concentrated, treated with water, and extracted with ether. This was washed with water, dried (Na_2SO_4), and concentrated to give an oil which was dissolved in ethanol (150 ml.) and water (30 ml.), and potassium cyanide (26 g., 0.4 mole) was added. The mixture was stirred at reflux for 42 hr. under nitrogen and then cooled, diluted with water, and extracted with ether which was dried (Na_2SO_4), concentrated, and distilled; yield 70–80%; ν_{max} 3450, 2150 cm.$^{-1}$. Most of the nitriles thus prepared were not distilled and were used without further purification, but 10-hydroxytridec-8-ynonitrile (7, R = C_3H_7; n = 6), b.p. 134–138° (0.5 mm.), was isolated.

Anal. Calcd. for $C_{13}H_{21}NO$: C, 75.4; H, 10.1; N, 6.8. Found: C, 75.5; H, 10.3; N, 7.0.

7-Hydroxydec-5-ynonitrile (7, R = C_3H_7; n = 6), b.p. 110–114° (0.35 mm.), was also isolated.

Anal. Calcd. for $C_{10}H_{15}NO$: C, 72.7; H, 9.1; N, 8.5. Found: C, 72.6; H, 9.1; N, 8.7.

Methyl Hydroxyalkynoates (8).—A solution of the nitrile (0.2 mole) and KOH (17 g.) in ethanol (100 ml.) and water (30 ml.) was kept at reflux for 64 hr. under nitrogen and then cooled, diluted with water, extracted with ether, made acidic with 2 *N* HCl (250 ml.), and extracted with ether. This was dried (Na_2SO_4) and concentrated to give an oil which was dissolved in methanol (60 ml.), and boron trifluoride (60% in methanol) (15 ml.) was added. The solution was kept at reflux under nitrogen for 1 hr. and then cooled, diluted with water, and extracted with ether. This was washed with saturated $NaHCO_3$, dried (Na_2SO_4), concentrated, and distilled; yield 70–80%; ν_{max} 3450, 2230, 1740 cm.$^{-1}$. In this way the acetylenic esters in Table I were prepared. Sometimes diazomethane was used for esterification.

Methyl Hydroxyalk-*cis*-enoates (9).—The acetylenic ester was dissolved in hexane (distilled from Raney nickel), Lindlar[21]

(18) All distillations were carried out under nitrogen. Infrared absorption was determined for thin films unless otherwise stated, using Model 137 Infracord.

(19) G. B. Bachman, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 323.

(20) E. R. H. Jones, L. Skatteböl, and M. C. Whiting, *J. Chem. Soc.*, 4765 (1956).

(21) H. Lindlar, *Helv. Chim. Acta*, **35**, 446 (1952).

catalyst was added, and the mixture was hydrogenated to 1 mole uptake. The solution was filtered, concentrated, and distilled; yield 85–90%; ν_{max} 3450, 1740, no band at 970 cm.$^{-1}$. In this way the *cis* esters in Table I were prepared.

Methyl Hydroxyalk-*trans*-enoates (10).—The acetylenic acid (0.05 mole) dissolved in dry tetrahydrofuran (100 ml.) in a cooled glass-lined autoclave was treated with lithium hydride (0.11 mole). After 1 hr. a solution of lithium (0.11 g.-atom) in liquid ammonia (200 ml.) was added. The mixture was kept at room temperature in the autoclave for 24 hr. The ammonia was then allowed to evaporate, and the residue was dissolved in water, acidified with 2 *N* HCl, and extracted with ether. This was dried (Na_2SO_4) and concentrated to give an oil which was esterified with diazomethane and distilled; yield 70–80%; ν_{max} 3450, 1740, 970 cm.$^{-1}$. In this way the *trans* esters in Table I were prepared. Use of boron trifluoride in methanol for esterification caused loss of hydroxyl and addition of methanol.[22]

12-Hydroxyheptadec-*trans*-10-enoic Acid (10, R = C_5H_{11}; n = 8).—The ester (0.5 g.), KOH (0.6 g.), ethanol (5 ml.), and water (2 ml.) were kept at reflux under nitrogen for 2 hr., cooled, acidified with 2 *N* HCl (10 ml.), and extracted with ether (500 ml.) which was dried and concentrated to give a solid which crystallized from petroleum ether (b.p. 40–60°) as rosettes, m.p. 42–43°; ν_{max} 3400, 1720, 965 cm.$^{-1}$.

Anal. Calcd. for $C_{17}H_{32}O_3$: C, 71.8; H, 11.3. Found: C, 72.1; H, 11.0.

7-Acetoxydec-5-ynoic Acid (13, R = C_3H_7).—The hydroxy acid (0.05 mole) was mixed with pyridine (6 ml.) and acetic anhydride (6 ml., 0.06 mole). The solution was kept for 18 hr. and then poured into water and extracted with ether which was washed with water, dried (Na_2SO_4), concentrated, and distilled to give 6.7 g. (60%) of an oil, b.p. 135° (0.35 mm.); ν_{max} 2240, 1745, 1720, 1340 cm.$^{-1}$.

Anal. Calcd. for $C_{12}H_{18}O_4$: C, 63.7; H, 8.0. Found: C, 63.8; H, 7.9.

Similarly prepared was 7-acetoxydec-*trans*-5-enoic acid, b.p. 110° (0.04 mm.); ν_{max} 1745, 1720, 1340, 965 cm.$^{-1}$.

Anal. Calcd. for $C_{12}H_{20}O_4$: C, 63.1; H, 8.8. Found: C, 63.3; H, 9.0.

Use of a greater excess of acetic anhydride caused mixed anhydride formation. Other acetoxy acids were similarly prepared without distillation and used without further purification.

7-Acetoxydec-5-ynoyl Chloride (14, R = C_3H_7).—The acetoxy acid (9 g., 0.04 mole) and oxalyl chloride (8.5 ml., 0.1 mole) were kept at reflux for 90 min. and then concentrated and distilled to give 8.1 g. (83%) of an oil, b.p. 104–105° (0.35 mm.); ν_{max} 2240, 1800, 1740, 1340 cm.$^{-1}$.

Anal. Calcd. for $C_{12}H_{17}ClO_3$: C, 58.9; H, 7.0; Cl, 14.5. Found: C, 58.6; H, 7.2; Cl, 14.2.

Similarly prepared was 7-acetoxydodec-5-ynoyl chloride (**14**, R = C_5H_{11}), b.p. 126–132° (0.4–0.6 mm.).

Anal. Calcd. for $C_{14}H_{21}ClO_3$: C, 62.2; H, 7.8; Cl, 13.1. Found: C, 61.6; H, 7.8; Cl, 13.0.

11,12,13-Trihydroxyoctadecanoic Acid.—13-Hydroxyoctadec-11-*cis*-enoic acid (0.21 g.) was added to osmium tetroxide (0.2 g.) and pyridine (0.15 ml.) in dry ether (15 ml.). After 2 hr. the ether was removed by evaporation and the residue was dissolved in dioxane. Hydrogen sulfide was passed through the solution for 10 min., the solution was filtered, concentrated, and the residue was dissolved in ethyl acetate. This was extracted with aqueous sodium carbonate which was acidified and extracted with ethyl acetate to give, on concentration, a solid (0.18 g.) which crystallized from ethyl acetate–ether; m.p. 100–104°; ν_{max}^{Nujol} 3400, 1715 cm.$^{-1}$.

Anal. Calcd. for $C_{18}H_{36}O_5$: C, 65.0; H, 10.9. Found: C, 65.0; H, 10.7.

1-Chloro-10,11-epoxyundecane.—1-Chloroundec-10-ene (34 g., 0.18 mole) was dissolved in dry ether (20 ml.) and 0.5 *M* ethereal monoperphthalic acid (380 ml., 0.19 mole) was added. When the concentration of peracid had fallen to 0.06 *N*, the solution was filtered, washed with saturated $NaHCO_3$, dried (Na_2SO_4), concentrated, and distilled to give 10 g. (27%) of an oil, b.p. 150–154° (12 mm.), showing no bands due to hydroxyl or terminal methylene in infrared absorption.

Anal. Calcd. for $C_{11}H_{21}ClO$: C, 64.6; H, 10.3. Found: C, 64.7; H, 10.3.

2-(7-Acetoxydec-5-ynoyl)cyclohexanone (15, R = C_2H_7; n = 5).—Morpholinocyclohexene (6.7 ml., 0.04 mole) and triethylamine (5.5 ml., 0.04 mole) in dry chloroform (25 ml.) were added over 20 min. to a solution of 7-acetoxydec-5-ynoyl chloride (7.8 g., 0.032 mole) in dry chloroform (25 ml.) at 50°. Stirring was continued at this temperature for 17 hr. then 20% sulfuric acid (20 ml.) was added, and stirring was continued at 70° for 6 hr. The aqueous layer was neutralized to pH 6 with 2 *N* NaOH and extracted with chloroform which was dried (Na_2SO_4), concentrated, and distilled to give 5.0 g. (51%) of an oil, b.p. 161–165° (0.3 mm.); ν_{max} 2230, 1740, *ca.* 1600, 1340 cm.$^{-1}$; purple color with ferric chloride.

Anal. Calcd. for $C_{18}H_{30}O_4$: C, 70.6; H, 8.5. Found: C, 69.8; H, 8.5.

Other diketones were similarly prepared without distillation and used without further purification. In similar conditions acetoxyl was lost from the analogous *trans* derivative.

Hydroxyoxoalkynoates (16).—The diketone (0.01 mole), KOH (0.03 mole), and water (20 ml.) were kept at reflux for 3 hr., cooled, acidified with 2 *N* HCl, and extracted with ether which was dried (Na_2SO_4) and concentrated to give an oil which was esterified with diazomethane and distilled: yield *ca.* 50%; ν_{max} 3500, 2220, 1740, 1720 cm.$^{-1}$. In this way methyl 13-hydroxy-7-oxohexadec-11-ynoate (**16**, R = C_3H_7; n = 5), b.p. 164–168° (0.2 mm.), was prepared.

Anal. Calcd. for $C_{17}H_{28}O_3$: C, 68.9; H, 9.5. Found: C, 68.7; H, 9.3.

Methyl 12-hydroxy-6-oxoheptadec-10-ynoate (**16**, R = C_5H_{11}, n = 4), b.p. 157° (0.15 mm.), was also prepared.

Anal. Calcd. for $C_{18}H_{30}O_4$: C, 69.6; H, 9.7. Found: C, 69.4; H, 9.4.

Methyl 12-Hydroxy-6-oxoheptadec-10-*cis*-enoate was prepared similarly, or by reduction of acetylenic ester: b.p. 148–150° (0.1 mm.); ν_{max} 3500, 1740, 1720 cm.$^{-1}$.

Anal. Calcd. for $C_{18}H_{32}O_4$: C, 69.2; H, 10.3. Found: C, 69.4; H, 10.4.

Methyl 6,12-Dihydroxyheptadec-10-ynoate (17, R = C_5H_{11}; n = 4).—Methyl 12-hydroxy-6-oxoheptadec-10-ynoate (0.93 g.) was dissolved in ethanol (12 ml.) and sodium borohydride (0.40 g.) in water (10 ml.) was added slowly with shaking. The mixture was kept for 24 hr., diluted, and extracted with ether. This was dried (Na_2SO_4), concentrated, and distilled to give 0.49 g. (53%) of an oil, b.p. 148–152° (0.04 mm.); ν_{max} 3400, 2240, 1750 (shoulder 1740) cm.$^{-1}$.

Anal. Calcd. for $C_{18}H_{32}O_4$: C, 69.2; H, 10.3; active H, 0.64; bound Grignard, 1.0 equiv. Found: C, 69.4; H, 10.2; active H, 0.72; bound Grignard, 1.2 equiv.

This compound resisted hydrogenation, probably because of its insolubility in suitable solvents.

Non-1-en-4-yn-6-ol.—Hex-1-yn-3-ol (11.2 ml., 0.1 mole) was added over 30 min. to a solution of ethylmagnesium bromide (from 25 ml. of bromoethane) in dry tetrahydrofuran (100 ml.). The mixture was stirred at 50° for 1 hr. and then cooled, and cuprous bromide (0.43 g.) was added. After 20 min. 1-bromoprop-2-ene (14 ml., 0.16 mole) was added over 1 hr. The mixture was kept at 80° for 8 hr., then decomposed with ice and 2 *N* H_2SO_4 (150 ml.), and extracted with ether which was washed with saturated $NaHCO_3$, dried (Na_2SO_4), concentrated, and distilled to give 8.3 g. (60%) of an oil, b.p. 102–103° (16 mm.); ν_{max} 3350, 2220 (weak), 1640, 990, 915 cm.$^{-1}$.

Anal. Calcd. for $C_9H_{14}O$: C, 78.2; H, 10.2. Found: C, 78.1; H, 10.2.

1,1-Diethoxyoct-3-yn-5-ol.—Ethylmagnesium bromide (from 11 ml. of bromoethane) in dry ether (100 ml.) was added over 1 hr. to 1,1-diethoxybut-3-yne[23] (16 ml., 0.1 mole) in dry ether (100 ml.). After 3 hr., butanal (9 ml., 0.1 mole) in dry ether (100 ml.) was added over 1 hr. The mixture was stirred for 16 hr. and then decomposed with 10% NH_4Cl (100 ml.) and extracted with ether which was dried (Na_2SO_4), concentrated, and distilled to give 14 g. (65%) of an oil, b.p. 82–86° (0.2 mm.); ν_{max} 3450, 2240, 1120, 1055, 1025 cm.$^{-1}$.

Anal. Calcd. for $C_{12}H_{22}O_3$: C, 67.3; H, 10.3. Found: C, 67.1; H, 10.1.

Non-*trans*-2-en-4-ol.—Crotonaldehyde (25 ml., 0.3 mole) in dry ether (100 ml.) was added over 1 hr. at 0° to the Grignard reagent from 1-bromopentane (45 ml., 0.3 mole) and magnesium (7.2 g.) in dry ether (250 ml.). Stirring was continued for 1 hr. and then the mixture was decomposed with saturated NH_4Cl and 2 *N* H_2SO_4, extracted with ether which was dried (Na_2SO_4),

(22) Cf. A. K. Lough, *Biochem. J.*, **90**, 40 (1964).

(23) L. Miginiac-Groizeleau, *Ann. Chim.* (Paris), **6**, 1071 (1961).

concentrated, and distilled to give 28.6 g. (68%) of an oil, b.p. 87–89° (12 mm.); ν_{max} 3400, 965 cm.$^{-1}$.

Anal. Calcd. for $C_9H_{18}O$: C, 76.0; H, 12.8. Found: C, 76.4; H, 12.6.

The acetate of this alcohol, prepared using acetic anhydride in pyridine, had b.p. 92–94° (13 mm.); ν_{max} 1740, 1675, 1240, 965 cm.$^{-1}$.

Anal. Calcd. for $C_{11}H_{20}O_2$: C, 71.7; H, 10.9. Found: C, 71.9; H, 11.1.

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Hypocholesterolemic Agents. V.[1a] Isomeric Azacholesterols[1b]

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The potent hypocholesterolemic activity of certain diaza analogs of cholesterol prompted the synthesis of a series of cholesterol isosteres having only one nitrogen atom in the side chain. The hypocholesterolemic activity of these isomers was examined and certain rationalizations regarding structure and activity were presented.

One approach to the development of hypocholesterolemic agents has been the synthesis of compounds which will inhibit the endogenous synthesis of cholesterol. In this connection, investigations by several groups[2] have demonstrated that feeding cholesterol to laboratory animals promptly suppresses hepatic cholesterol synthesis. More recently, this negative feedback control of cholesterogenesis was found to be operative in man as well.[3]

Previous publications[4,5] from these laboratories described various diaza analogs of cholesterol which were synthesized as part of a program aimed at finding substances which would simulate cholesterol in the feedback mechanism. In these studies 20,25-diazacholesterol (X) was noted to be an extremely potent inhibitor of cholesterol synthesis in laboratory animals.[5] Subsequent clinical studies with this agent confirmed the high order of hypocholesterolemic activity in humans.[6]

Additional structure–activity relationship studies with the diazacholesterols tended to support the contention that these compounds were suppressing cholesterol synthesis in a "cholesteromimetic" fashion.[4,5] For example, replacing the isosteric dimethylamino end group with bulkier substituted amines markedly reduced the hypocholesterolemic activity. Lengthening the side chain by inserting one methylene group between the nitrogen atoms produced a similar effect. Shortening the side chain by one methylene group, however, produced little change in activity. These results implied that a receptor site with dimensions specific for cholesterol was involved and one must have an accurate fit of the substrate in order to get maximum activity. Accordingly, any structural change which tended to impede adsorption of the substrate molecule at the receptor site, produced a corresponding decrease in the hypocholesterolemic activity.

In an effort to obtain further insight as to the mode of action of these compounds, a series of cholesterol isosteres having only one nitrogen atom in the side chain was synthesized and biologically evaluated. Because of the disposition of the nitrogen atoms in the diazacholesterols described above, it was possible that these substances were exerting their hypocholesterolemic action *via* an intramolecular metal-chelating process which would tie up certain trace metals essential for cholesterol biosynthesis. Such a mechanism was proposed by Curran[7] to explain the inhibitory action of 8-quinolinol. The azacholesterols, on the other hand, would be incapable of acting in this manner. In addition, it was hoped that the study of the isomeric azacholesterols would provide further information regarding the electrical and topographical features of the receptor site.

20-Azacholesterol (IIIb, N-isohexyl-N-methyl-17β-aminoandrost-5-en-3β-ol) was readily obtained in several steps from 3β-acetoxyandrost-5-en-17-one (I). Condensation of I with isohexylamine in the presence of a catalytic amount of *p*-toluenesulfonic acid gave the expected 17-imine (II) as an oil. Reduction of II with lithium aluminum hydride afforded the corresponding amine (IIIa) which was methylated under Eschwiler–Clarke conditions[8] to give IIIb. Infrared and n.m.r.[9] analysis of the product clearly showed the characteristic absorption band for the N-methyl group at 3.6 μ[10] and 128 c.p.s.,[11] respectively.

Surprisingly, several attempts to carry out a Leuckart reductive amination of I with isohexylamine were unsuccessful. This was in marked contrast with the ease

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