The yield improved steadily up to four hours, but further refluxing had little effect.

EXPERIMENTAL[10]

Experimental details are given only for the two previously undescribed esters. Similar procedures were used for the others.

n-Heptyl phenylacetate. Phenylacetonitrile (46.8 g., 0.40 mole), *n*-heptyl alcohol (46.5 g., 0.40 mole), and *p*-toluenesulfonic acid monohydrate (76.0 g., 0.40 mole, including 0.40 mole water) were refluxed with stirring for 6 hr. The addition of 100 ml. of water dissolved the ammonium salts, and caused separation into two layers. The ester layer was separated, washed with 20% sodium carbonate solution, dried over anhydrous magnesium sulfate, and distilled from a Claisen flask. Yield of crude ester, 66.0 g., 0.28 mole.

The crude ester was washed with 5% sodium carbonate solution, and 200 ml. of toluene was added. This was distilled off at atmospheric pressure for azeotropic removal of water. After removal of water, the ester was distilled through a 60-cm. tantalum wire spiral column, using a total reflux partial take-off head. The fraction of constant index of refraction boiled at 146–147° at 2.0 mm., and totaled 31.4 g. The neutralization number as determined with alcoholic potassium hydroxide was zero. Other properties are given in Table II.

Anal. Calcd. for $C_{15}H_{22}O_2$: C, 76.88; H, 9.47. Found: C, 77.06; H, 9.79.

Di-n-propyl-β,β′-oxydipropionate. β,β′-Oxydipropionitrile (37.2 g., 0.30 mole), *n*-propyl alcohol (36.0 g., 0.60 mole), and benzenesulfonic acid monohydrate (105.6 g., 0.60 mole, including 0.60 mole of water) were refluxed with stirring for 6 hr., and the reaction mixture worked up as described above. The average yield of two runs was 18.6 g., 0.075 mole. Purification of the crude ester was performed as described above. Distillation of 21.7 g. of crude ester gave 9.1 g. of purified ester boiling at 146–147° at 2.0 mm. The neutralization number with alcoholic potassium hydroxide was almost zero. Other properties are given in Table II.

Anal. Calcd. for $C_{12}H_{22}O_5$: C, 58.51; H, 9.00. Found: C, 56.75; H, 8.65.

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(10) Analyses by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

Synthesis of a Steroidal Nitrogen Mustard[1]

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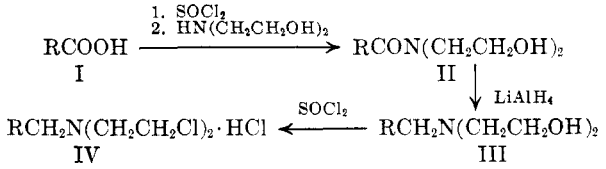
The nitrogen mustards are recognized as anticancer agents.[2] We wish to record the synthesis of a steroid-nitrogen mustard combination,[3] which was expected to have transport characteristics considerably different from those of the more familiar low-molecular weight nitrogen mustards. It was believed possible that such difference could reflect in enhanced activity.

The starting point in the synthesis was 5-cholestene-3β-carboxylic acid (I)[4] obtained from cholesteryl chloride by carbonation of the Grignard reagent.[5] Treatment with thionyl chloride followed by reaction of the acid chloride with diethanolamine gave the *N,N*-bis(hydroxyethyl) derivative



R = 3β-cholesteryl

II of 5-cholestene-3β-carboxamide. Lithium aluminum hydride, by reducing the amide grouping in II to amine, formed 3β-[bis(hydroxyethyl)aminomethyl]-5-cholestene(III).[6] To generate the nitrogen mustard, both hydroxyl groups were replaced with chlorine with help of thionyl chloride. The hydrochloride of the tertiary amine III as well as the hydrochloride IV of the nitrogen mustard were also prepared.

The nitrogen mustard was made available for assay to Drs. H. M. Lemon and H. H. Wotiz at Boston University Medical School who very kindly submitted the following report:

"The relative insolubility of the steroid-mustard in the conventionally used solvents for injection made the toxicity study extremely difficult. Nevertheless, preliminary studies were carried out by intraperitoneal administration of a suspension of the mustard in a starch solution.

The survival rates of mice, guinea pigs, or rats did not differ significantly from the controls following doses of 34, 250, 500, and 1000 mg./kg. Histopathological examination of the mouse livers showed evidence of plastic peritonitis, yellow atrophy, and multinucleated giant cells. The mouse kidneys showed evidence of interstitial hemorrhagic nephritis at the border of the cortex and medulla. Because of its extreme insolubility it is impossible to tell whether these effects were caused by the inherent toxicity of the mustard or by a local irritating effect due to its limited absorption from the peritoneum."

(1) These studies were aided by grants from the American Cancer Society and from the National Institutes of Health, and by an Institutional Research Grant from the American Cancer Society.

(2) *Cf.* A. Gellhorn, *Cancer Research*, **13**, 205 (1953); J. P. Greenstein, *Biochemistry of Cancer*, 2nd ed., Academic Press, Inc., New York, N. Y., 1954, p. 282.

(3) Compare (a) G. R. Vavasour, H. I. Bolker, and A. F. McKay, *Can. J. Chem.*, **30**, 933 (1952); (b) G. G. Hazen, Doctoral dissertation, University of Michigan, 1951, *Chem. Abstr.*, **47**, 8761 (1953). For the incorporation of a carcinogenic hydrocarbon in nitrogen mustard compounds see O. M. Friedman and A. M. Seligman, *J. Am. Chem. Soc.*, **70**, 3082 (1948).

(4) The orientation at the 3-position was shown by E. J. Corey and R. A. Sneen, *J. Am. Chem. Soc.*, **75**, 6234 (1953); G. Roberts, C. W. Shoppee, and R. J. Stephenson, *J. Chem. Soc.*, 2705 (1954).

(5) *Cf.* R. H. Baker and E. N. Squire, *J. Am. Chem. Soc.*, **70**, 1487 (1948).

(6) A related sequence was reported starting with Δ^5-3β-acetoxybisnorcholenic acid and giving the corresponding di-(hydroxyethyl)amine derivative. H. L. Herzog, C. C. Payne, and E. B. Hershberg, *J. Am. Chem. Soc.*, **77**, 5324 (1955).

EXPERIMENTAL[7]

5-Cholestene-3β-carboxylic acid (I). The necessary starting material was prepared by the reaction of cholesterol and thionyl chloride.[3a] The vacuum-dried cholesteryl chloride, after several crystallizations from absolute alcohol, showed m.p. 94.5–95° and $[\alpha]_D^{24.5}$ −33°. The yield was 61%. The constants recorded before for cholesteryl chloride are m.p. 95–96°[3a] and 96–97°[5], and $[\alpha]_D^{25}$ −26.4°[3a]. Grignardation and carbonation of cholesteryl chloride was performed by a modification of the method of Baker and Squire.[5]

A mixture of 3.39 g. (0.14 g.-atoms) of turnings of sublimed magnesium, 7.6 g. (0.070 mole) of dry ethyl bromide, and absolute ether was magnetically stirred in a three-necked flask that had been scrupulously dried and fitted with a condenser and dropping funnel. Dry nitrogen was used to blanket the reaction mixture throughout the experiment. The dropwise addition of a solution of 28.2 g. (0.069 mole) of cholesteryl chloride in 85 ml. of dry ether was begun at the onset of the vigorous reaction between the ethyl bromide and magnesium, and was completed over the course of 3 hr. During the addition and for 46 hr. thereafter, the reaction mixture was warmed to maintain a gentle reflux. At the end of this period the solution contained a gray-white precipitate; some unreacted magnesium was also apparent.

Dry carbon dioxide was bubbled into the vigorously stirred Grignard mixture for 12 hr. Dry ether was added at intervals to keep the volume close to 400 ml. The carbonation mixture was poured over 200 ml. of cold 10% sulfuric acid solution. The ether layer was filtered and the insoluble material was rinsed with several portions of ether. The combined ether solutions were dried with sodium sulfate, and then warmed on the steam bath to remove solvent. The viscous residue, after crystallization from benzene, gave 14.0 g. (49%) of white crystalline 5-cholestene-3β-carboxylic acid, m.p. 216–217°, $[\alpha]_D^{23.5}$ −13°. The values given before are, *inter alia*, m.p. 218–220°, 225–227°, and 226–227° (to opaque melt)[4,5], and $[\alpha]_D$ −10°[4] and $[\alpha]_D^{24}$ −14°.[8]

N,N-Bis(hydroxyethyl)-5-cholestene-3β-carboxamide (II). 5-Cholestene-3β-carbonyl chloride was prepared by boiling a solution of 8.41 g. (0.020 mole) of 5-cholestene-3β-carboxylic acid and 15 g. (0.13 mole) of thionyl chloride in 30 ml. of sodium-dried benzene for 5.5 hr. The reaction mixture was then allowed to stand overnight at room temperature. Exposure to reduced pressure at steam bath temperatures removed volatile materials and left the tan-colored acid chloride, which was washed with cold benzene and collected by filtration.

A homogeneous mixture of the acid chloride in 50 ml. of dry benzene containing 13.3 g. (0.13 mole) of dry diethanolamine was stirred and heated in an oil bath at 63° for 4 hr. The solid deposited on allowing the reaction mixture to stand overnight was collected, and was crystallized from methanol (*ca.* 40 ml.). The mother liquors were concentrated under reduced pressure on the steam bath, and water was added to the viscous residue. The resulting solid was collected by filtration and was crystallized from methanol. The two crops of crystals, combined and dried over potassium hydroxide pellets *in vacuo*, weighed 5.6 g. (56%) and showed an indefinite melting point with softening at 168° and complete liquefaction at approximately 190°. A sample of this product, after two crystallizations from methanol and vacuum drying, afforded *N,N*-bis(hydroxyethyl)-5-cholestene-3β-carboxamide, m.p. 182–189°, $[\alpha]_D^{24.5}$ −10°.

Anal. Calcd. for $C_{32}H_{55}O_3N$: N, 2.8. Found: N, 2.7.

The material as a mull with mineral oil showed an infrared absorption peak at 6.22 μ.

3β-[Bis(hydroxyethyl)aminomethyl]-5-cholestene (III). A mixture of amide II (5.61 g. or 0.11 mole), 5.0 g. (0.13 mole) of lithium aluminum hydride, and 100 ml. of ether was magnetically stirred at room temperature for 24 hr. Water was added dropwise, slowly and with stirring, followed by 700 ml. of a concentrated solution of sodium potassium tartrate and ammonium sulfate. The ether solution was removed, and the turbid aqueous layer was extracted with 3 portions of chloroform. The combined ether and chloroform solutions were dried with sodium sulfate, and all volatile material was removed by distillation on the steam bath at water pump pressure. The residual 3β-[bis(hydroxyethyl)aminomethyl]-5-cholestene, m.p. 119–124°, weighed 4.1 g. (76%). The analytical sample, prepared by three crystallizations from absolute alcohol, melted at 135–137.5° and showed $[\alpha]_D^{23.8}$ −24°.

Anal. Calcd. for $C_{32}H_{57}O_2N$: C, 78.8; H, 11.8; N, 2.9. Found: C, 79.3; H, 11.6; N, 3.1.

No absorption at 6.22 μ was evident.

The hydrochloride of amine III was prepared by saturating an acetone solution of the amine with dry gaseous hydrogen chloride. The white precipitate was collected, crystallized from acetone, and dried in high vacuum at 110°. The salt melted at 207.5° (dec.) with preliminary softening.

Anal. Calcd. for $C_{32}H_{58}O_2NCl$: Cl, 6.8. Found: Cl, 7.0.

3β-[Bis(chlorethyl)aminomethyl]-5-cholestene hydrochloride (IV). Thionyl chloride (8 g.; 0.07 mole) was added in 1 portion to a hot solution of 4.1 g. (0.085 mole) of amine III in 100 ml. of dry benzene. The mixture, in which a thick gelatinous precipitate had formed, was heated on the steam bath for 35 min. Volatile material was removed by vacuum distillation on the steam bath, and the brown residue was dried *in vacuo*. A hot chloroform solution of the crude product was treated with decolorizing carbon (Norit), the chloroform was removed by vacuum distillation, and the residue was crystallized from 150 ml. of absolute alcohol. The resulting tan crystals, after air-drying, weighed 3.4 g. (70%) and showed m.p. 203–210° (dec.) with preliminary softening. The melting point of salt IV, as well as of all the others, was not sharp, coloration and decomposition being evident. Three crystallizations of the product from absolute ethanol brought the melting point to 198.5–203°. In another experiment, crystallization once from methanol and twice from acetone followed by vacuum drying at 110° furnished hydrochloride IV with m.p. 196–201° (dec.) and $[\alpha]_D^{20.4}$ −17°.

Anal. Calcd. for $C_{32}H_{56}NCl_3$: Cl, 19.0. Found: Cl, 18.7.

In another preparation, the hydrochloride after recrystallization three times from alcohol and drying at 100° *in vacuo* had m.p. 192–194° (dec.), $[\alpha]_D^{26.5}$ −19°.

Anal. Calcd. for $C_{32}H_{56}NCl_3$: C, 68.5; H, 10.1; N, 2.5; Cl, 19.0. Found: C, 68.7; H, 10.1; N, 2.6; Cl, 18.8.

3β-[Bis(chloroethyl)aminomethyl]-5-cholestene. A sample of hydrochloride IV dissolved in chloroform was shaken with 10% potassium hydroxide solution. The chloroform solution was washed with water, dried with sodium sulfate, and all solvent was removed. The residual yellow oil was dissolved in acetone, and methanol was added until the solution was cloudy. Cooling resulted in the precipitation of white crystalline 3β-[bis(chloroethyl)aminomethyl]-5-cholestene (m.p. 67.5–70°), which after two crystallizations from absolute alcohol and drying *in vacuo* at 57°, showed m.p. 72–75°.

Anal. Calcd. for $C_{32}H_{55}NCl_2$: Cl, 13.5. Found: Cl, 13.3.

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(7) Melting points are uncorrected. Optical rotations were taken with 1% solutions in chloroform. The elementary analyses were performed by Carol K. Fitz, 115 Lexington Avenue, Needham Heights 94, Mass.

(8) R. H. Baker and E. N. Squire, *J. Am. Chem. Soc.*, 70, 4134 (1948).