STEROIDS

II. REACTION OF AMINES WITH CHOLESTERYL CHLOROFORMATE AND PYROLYSIS OF N-BENZYL CHOLESTERYL CARBAMATE[1]

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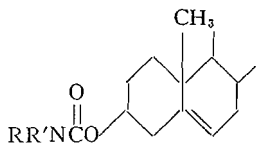
ABSTRACT

Cholesteryl chloroformate can be used to isolate small amounts of amines from aqueous solutions as N-substituted cholesteryl carbamates. These derivatives are easily isolated and identified. Their possible use for the identification of amines obtained in degradative studies is discussed. The thermal decomposition of N-benzyl cholesteryl carbamate gives carbon dioxide, cholesterol, cholest-3,5-diene, and *sym*-dibenzylurea. A mechanism of formation of these products is discussed.

During the course of a previous investigation (5) of the preparation of N-substituted-3-aminocholest-5-enes it was noted that Bergmann and Haskelberg (1) had reported the formation of 3-anilinocholest-5-ene by heating cholesteryl chloroformate with methylaniline. If this latter reaction had occurred it would have offered a simple method of preparation of N-substituted-3-aminocholest-5-enes. However, all attempts to repeat this work were unsuccessful. On the other hand, it was found that cholesteryl chloroformate combines with primary and secondary amines to give practically quantitative yields of N-substituted cholesteryl carbamates (I). In this manner Verdino and Schadendorff (6) and Kucherov and Kocheshkov (2) have prepared a



I

number of N-aryl cholesteryl carbamates. This method has been changed to recover amines [as the N-substituted cholesteryl carbamates (cf. Table I)] from their dilute aqueous solutions. Since these derivatives are formed rapidly and with ease this procedure provides an excellent method for the identification of biologically important amines often obtained in dilute solution during degradation studies. Also the large increase in molecular weight in transforming methyl-, dimethyl-, ethylamines, etc., into the corresponding N-substituted cholesteryl carbamates is a decided advantage. Thus it is considered that these derivatives will be a distinct asset in assisting in the identification of amines from biological sources.

Wieland, Honold, and Vila (7) first reported the preparation of cholesteryl chloroformate and its thermal transformation to 3-β-chlorocholest-5-ene. The melting point of their product was below the accepted melting point of pure 3-β-chlorocholest-5-ene so this work was repeated. It was found that

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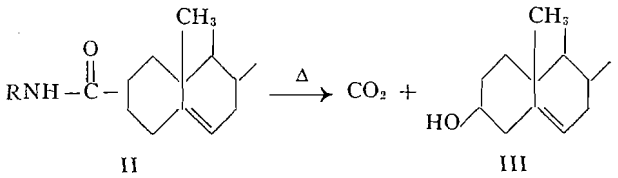
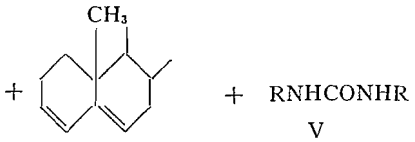
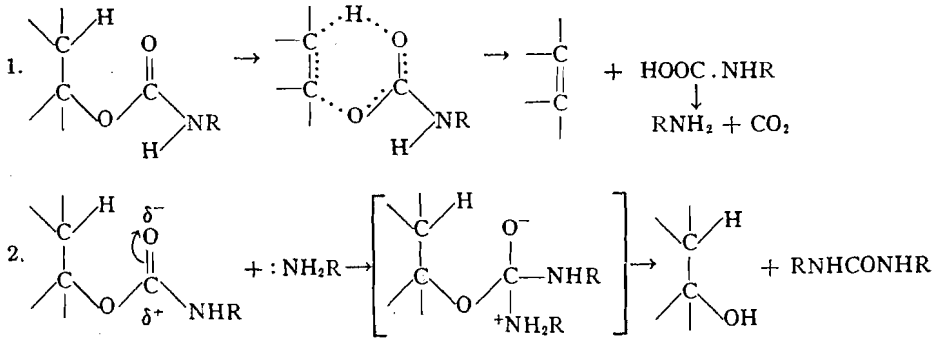
pyrolysis of cholesteryl chloroformate at 150° C. gives a quantitative yield of carbon dioxide and about 20% hydrogen chloride along with cholesteryl chloride contaminated with unsaturated hydrocarbon. Pure cholesteryl chloride (m.p. 95–96° C.) could only be obtained by chromatographing on alumina. The unsaturated hydrocarbon fractions were not identified. In the pyrolysis of N-benzyl cholesteryl carbamate, cholest-3,5-diene (IV) was identified in the unsaturated hydrocarbon fraction (*vide infra*).

In order to be certain that no rearrangement of N-substituted cholesteryl carbamates to 3-aminocholest-5-enes occurs under any conditions of heating, the thermal decomposition of N-benzyl cholesteryl carbamate (II) was studied in detail. No 3- or 6-benzylaminocholest-5-enes were present in the degradation products of any of the several runs. Instead the products were identified as carbon dioxide, 3-β-hydroxycholest-5-ene (cholesterol) III, cholest-3,5-diene (IV), and *sym*-dibenzylurea. Generally a small quantity (1–10%) of the N-benzyl cholesteryl carbamate (II) was recovered unchanged.





If the thermal decomposition of N-benzyl cholesteryl carbamate occurred by the same mechanism as the Chugaev reaction (the thermal decomposition of xanthate esters to produce olefins) then one would expect a good yield of cholest-3,5-diene and no cholesterol [cf. the thermal decomposition of cholesteryl methyl xanthate (3)]. The formation of cholesterol and cholest-3,5-diene together in the pyrolysis of N-benzyl cholesteryl carbamate suggests that two reactions are occurring as follows.



The first reaction, which is pictured with the cyclic transition state similar to that used for the thermal decomposition of cholesteryl methyl xanthate by O'Connor and Nace (3), would produce cholest-3,5-diene, carbon dioxide, and benzylamine, while reaction by the second path would give cholesterol and *sym*-dibenzylurea. If it is considered (without support other than that carbon dioxide is produced in 50% yield and its measurement is quantitative) that both these reactions occur to approximately the same extent, then the products have been obtained from the thermal decomposition of N-benzyl cholesteryl carbamate in the following yields; carbon dioxide (102%), cholesterol (86%), *sym*-dibenzylurea (77%), cholest-3,5-diene (20–40%). The low yields of *sym*-dibenzylurea may be due to partial volatilization of the benzylamine before complete reaction occurred. This is partly corroborated by the slight difference in weight between the weights of starting material and the weights of carbon dioxide plus nonvolatile products. The hydrocarbon, cholest-3,5-diene, was the most difficult product to account for quantitatively. This could be caused by treatment of the ether solution with hydrogen chloride to substantiate the absence of 3- or 6-benzylaminocholest-5-enes.

EXPERIMENTAL[2]

Cholesteryl Chloroformate

Fifty grams of cholesterol was converted into cholesteryl chloroformate (m.p. 119.5–121° C., $[\alpha]_D^{24}$ −26.7) in 86% yield using essentially the method of Wieland *et al.* (7). The acetone mother liquor from the crystallized cholesteryl chloroformate gave a second crop which melted 117.5–119° C. The total yield was 92%.

N-Methyl-N-phenyl Cholesteryl Carbamate

Cholesteryl chloroformate (2.0 gm., 0.004 mole) and methylaniline in 25 cc. of dry acetone were refluxed for two hours. After the solution was cooled and partially evaporated two crops of crystals (m.p. 127.5–131° C.) were obtained, yield 2.35 gm. (100%). One crystallization from acetone raised the melting point to 131–133° C., $[\alpha]_D^{24}$ −6.8. Calc. for $C_{35}H_{53}NO_2$: C, 80.90; H, 10.28; N, 2.69%. Found: C, 80.86; H, 10.36; N, 2.78%.

Treatment of Cholesteryl Chloroformate with Methylaniline

Two grams (0.004 mole) of cholesteryl chloroformate and 1.3 gm. (0.012 mole) of methylaniline on mixing together evolved much heat and crystals were deposited. This mixture was refluxed for three hours under nitrogen. The reaction mixture became homogeneous after 90 min. of heating. The cooled solution was poured into 100 cc. of 10% hydrochloric acid solution after which a very tacky gum separated. This gum was dissolved in hot *n*-butanol as described by Bergmann and Haskelberg (1) for the isolation of cholesteryl aniline. However, although several runs were made, no cholesteryl

[2] *All melting points were determined on a Kofler block. Chloroform was used as a solvent for the optical rotation determinations. Microanalyses were performed by C. W. Beazley, Skokie, Illinois.*

aniline could be obtained from the gum. In some cases a few milligrams of dark solid, which melted at 75–105° C. were obtained.

N-Benzyl Cholesteryl Carbamate

N-Benzyl cholesteryl carbamate (m.p. 148.5–149.5° C., $[\alpha]_D^{24.6}$ −18.7°) was prepared in 89% yield by the method described above for the preparation of N-methyl-N-phenyl cholesteryl carbamate. The melting point reported in the literature (6) for N-benzyl cholesteryl carbamate is 148° C.

N-Alkyl Cholesteryl Carbamates

Method A.—Two mole equivalents of an alkylamine were added to cholesteryl chloroformate dissolved in ether. The heat of reaction was sufficient to reflux the ether. At the end of one hour the lumps of amine hydrochloride were broken up and removed by filtration and washed with ether. The ethereal filtrate and washings on evaporation to dryness gave practically quantitative yields of the N-alkyl cholesteryl carbamates. Generally one crystallization from alcohol was sufficient for purification.

Method B.—Cholesteryl chloroformate (1.0 gm., 0.002 mole) was dissolved in 100 cc. of ether and shaken with 1% aqueous amine (two mole equivalents) solution. The carbamates were isolated by evaporation of the water-washed ether solutions (N-methyl cholesteryl carbamate, which was insoluble in ether, was filtered off, and washed with ether). The crude products were purified by one crystallization from either acetone or 95% ethanol. The N-alkyl cholesteryl carbamates prepared by methods *A* and *B* are listed in Table I.

TABLE I

N-SUBSTITUTED CHOLESTERYL CARBAMATES

Substituents	Method	Yield, %	M.p., °C.	$[\alpha]_D^{24}$	Formula	Carbon		Hydrogen		Nitrogen	
						Calc'd.	Found	Calc'd.	Found	Calc'd.	Found
Methyl	B	95	204–205	−33.0	$C_{29}H_{49}NO_2$	78.50	78.76	11.13	11.20	3.16	2.95
Dimethyl	B	95	112–113	−27.1	$C_{30}H_{51}NO_2$	78.71	78.85	11.24	11.44	3.06	2.77
Ethyl	B	94	161.5–162	−29.0	$C_{30}H_{51}NO_2$	78.71	78.70	11.24	11.38	3.06	2.63
n-Propyl	B	95	141–142	−26.2	$C_{31}H_{53}NO_2$	78.93	78.85	11.33	11.34	2.97	2.74
Isopropyl	B	95	147.5–148.5	−26.8	$C_{31}H_{53}NO_2$	78.93	78.87	11.33	11.36	2.97	2.82
Allyl	B	91	142–143	−23.5	$C_{31}H_{51}NO_2$	79.25	79.05	10.94	11.26	2.98	2.58
n-Butyl	A	99	128–129	−27.6	$C_{32}H_{55}NO_2$	79.11	79.21	11.41	11.32	2.88	3.20
Isoamyl	B	100	128–130	−23.7	$C_{33}H_{57}NO_2$	79.29	79.13	11.49	11.42	2.80	2.70

Pyrolysis of Cholesteryl Chloroformate

Four grams (0.009 mole) of cholesteryl chloroformate were intimately mixed with a 10-fold quantity of finely ground Pyrex glass and placed in a flask. After all the air had been displaced from the apparatus with nitrogen, the outlet was connected to a gas absorption train containing a silver nitrate–dilute nitric acid solution in a wash-tower, a U-tube of Drierite, and a weighed U-tube charged with Ascarite and magnesium chlorate. The flask containing the cholesteryl chloroformate was placed in an oil bath preheated to 160° C. and the temperature was maintained at 150 ± 5° C. for two hours. A slow stream of nitrogen was introduced into the apparatus during the reaction

period. At the end of this time 0.375 gm. (96%) of carbon dioxide had been evolved and 0.248 gm. (equivalent to 20% of the chlorine of cholesteryl chloroformate being evolved as hydrogen chloride) of silver chloride was precipitated.

The residual mixture of glass and pyrolysis product was refluxed with acetone (50 cc.), filtered while hot, and the glass washed with ether. Evaporation of the solvents gave 3.5 gm. of solid (m.p. 73–79° C.). A portion (0.505 gm.) of this product was chromatographed on 20 gm. of alumina in a 24 mm. tube using 50 ml. portions of *n*-pentane as the eluant. It was found necessary to run a second chromatogram on this material. This procedure gave 212.2 mgm. (40.7%) of crude 3-β-chlorocholest-5-ene (m.p. 91–95° C.). One crystallization from acetone raised the melting point to 94.5–95.5° C., yield 90 mgm. This product did not depress the melting point of an authentic sample of 3-β-chlorocholest-5-ene. The remaining fractions from the chromatograms were oils or oily solids.

Thermal Decomposition of N-Benzyl Cholesteryl Carbamate

This was carried out in the same way as the decomposition of cholesteryl chloroformate. Forty grams of finely powdered Pyrex (to pass 28 mesh) and 4.0 gm. (0.0077 mole) of N-benzyl cholesteryl carbamate were intimately mixed and heated together for one hour at 290 ± 10° C. by immersion in a potassium nitrate – sodium nitrite bath. During the heating 0.174 gm. (51%) of carbon dioxide was evolved. After cooling, the organic material was extracted from the glass with hot chloroform and alcohol in turn. These solutions were combined, taken to dryness under nitrogen, and finally dried in a vacuum desiccator. The tan solid (3.8 gm.) was triturated with 50 cc. of absolute ether and the insoluble material recovered, yield 0.68 gm. (74%). This solid melted at 170–171° C. alone and on admixture with an authentic sample of *sym*-dibenzylurea. The ethereal filtrate was shaken with 10% hydrochloric acid and washed with 10% sodium bicarbonate solution and water. The organic layer was evaporated under nitrogen and dried *in vacuo*. This residue on treatment with 50 cc. *n*-pentane gave a white solid, yield 0.85 gm. (m.p. 124–141° C.). Treatment of this solid in the usual manner with digitonin gave a digitonide from which 0.54 gm. of cholesterol (m.p. 144.5–146.5° C.) was recovered. One recrystallization from 95% alcohol raised its melting point to 147.5–148.5° C. which was not depressed by mixture melting point with authentic cholesterol. The filtrate from the digitonide was treated to remove excess digitonin and then taken to dryness. Trituration of the residue with petroleum ether (30–60° C.) yielded a further 0.02 gm. (2.1%) of *sym*-dibenzylurea (m.p. 170.5–171.5° C.) and 0.03 gm. of unchanged N-benzyl cholesteryl carbamate (m.p. 147.5–148.5° C.) which was identified by mixed melting point.

The pentane solution was taken to dryness leaving a residue of 2.1 gm. This was redissolved in 50 cc. pentane and an aliquot containing 1.0 gm. chromatographed on 30 gm. of alumina (Alcoa, F-20, neutralized and reactivated). This column was washed with 100 cc. portions of solvent (cf. Table II).

TABLE II

LIQUID CHROMATOGRAM

Fraction No.	Solvent*	Residue (mgm.)	M.p. (°C.)
1	P	193.7 (557.9)**	70–74 (70.5–75.5)**
2	P	61.8 (26.9)**	Oil (67.5–70.0)**
3–11	P, P.E. to B	35.0	Oils
12–17	B	153.2	Semisolids
18–21	B–E (9: 1)	195.7	From 115° to 137°
22	B–E (9: 1)	38.6	133–140°
23–29	B–E (9: 1 to 1: 1)	163.5	125–135°
30–39	E, E–C, C–A (9: 1 to 1: 1)	207.9	Dark oils

* *P = pentane, P.E. = pet. ether (65–68°), B = benzene, E = ether, C = chloroform, A = acetone.*

** *The values in parentheses refer to another run made essentially to obtain a better value on the yield of cholest-3,5-diene.*

Fraction 1 on crystallization from 5.3 cc. of ethyl acetate – methanol (3: 1) yielded 390 mgm. of crystals (m.p. 78.5–80.0° C., $[\alpha]_D^{23}$ −112°). Another recrystallization gave material melting at 78.5–79.5° C., $[\alpha]_D^{23}$ −116 and $\lambda_{max}^{alc.}$ 235 mμ. Anal. calc. for $C_{27}H_{44}$: C, 87.97; H, 12.03%. Found: C, 87.83; H, 12.44%. The constants reported for cholest-3,5-diene are m.p. 80–81° C., $[\alpha]_D^{20}$ −129.6 (4), λ_{max} 235 mμ (8).

Fraction 2 recrystallized in the same manner gave 9 mgm. of solid which melted at 73–78° C.

Fractions 3–11 and 30–39 resisted all attempts at purification.

Fractions 12–17 were recrystallized from acetone to give 32 mgm. of N-benzyl cholesteryl carbamate (m.p. 145–147° C.) identified by mixture melting point.

Fractions 18–29 were also recrystallized from acetone to yield 123.4 mgm. of cholesterol (m.p. 147.5–149.0° C.) which was identified by a mixture melting point with authentic cholesterol.

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