Soluble Steroids II

Amino Acid Derivatives

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A series of amino acid derivatives of cholesterol, 9α-fluorohydrocortisone, 6α-fluoro-16α-hydroxycortisone-16,17-acetonide, and 2α-methyl-5α-dihydrotestosterone have been synthesized in a further attempt to provide compounds with increased aqueous solubility. The acylable hydroxy group of the steroid was converted to the chloroformate and was then condensed with the appropriate amino acid. The potassium salts of the various derivatives which have been prepared possess 100–600 times the aqueous solubility of the parent compounds. Infrared characteristics of the several compounds have been determined. Pharmacological studies have been undertaken and show, in general, reduced activity when the derivatives are administered subcutaneously.

THERE ARE two general methods by which the aqueous solubility of steroids has been increased. The first method is the addition of an adjunct; the second method is the preparation of derivatives having greater water solubility than the parent steroid. Of the many modifications that have been reported, the preparation of carbamates have been mentioned in only a few cases. In the first paper in this series (1) glucamine, glucosamine, and *N*-methylglucamine carbamates of various steroids were prepared. These derivatives possessed eight to 13 times the aqueous solubility of the parent compounds. Thus, it was felt that a series of steroids containing amino acid moieties, capable of salt formation, should give even greater water solubility. The slow hydrolysis of carbamates should give depot solutions of the various steroids.

The references to the use of amino acids to solubilize steroids are found primarily in the patent literature. A British and a Dutch patent describe the preparation of various steroid esters of amino acids and their treatment with hydrohalides to give compounds which are readily soluble in water (2, 3). Another patent covers salts of amino acid esters as well as diethylamine, piperidine, morpholine, and piperazine carbamates of hydrocortisone (4).

Amino acids have been used to increase the water solubility of antibiotics (5). When methionine, glutamic acid, or their *N*-acyl derivatives were allowed to react with streptomycin, dihydrostreptomycin, neomycin, viomycin, or other basic antibiotics, the toxicity of the antibiotics was reduced while the water solubility increased.

The effect of the presence of carbamate or carbonate moieties on the pharmacological activity of steroids has been reported by Brown and coworkers (6). They reported that *N*-alkyl 21-carbamates of corticosteroids had local but no systemic activity, while the 21-ethylcarbonate of hydrocortisone had systemic activity comparable to the free alcohol.

The present investigation is thus intended to make available for pharmacological testing various steroids containing amino acid moieties, such as glycine, phenylalanine, and glutamine.

DISCUSSION

Cholesterol was used as the model steroid for preliminary investigation because of its cost and ease of conversion to its chloroformate. The chloroformate was prepared according to the method described by Wieland, Honold, and Pascual-Vila (7). The chloroformate was then refluxed with an excess of glycine in a dioxane-water mixture. The reaction was completed in 4 hours, and the product, 3β-(5-cholestenyl)*N*-glycylcarbamate, was isolated. Carbon-hydrogen analyses on recrystallized samples of this compound indicated that the desired derivative had been obtained. The glycine derivative was also successfully prepared by refluxing an acetone-water mixture of the starting materials for 4 hours. However, with pyridine as the solvent, only 3β-(5-cholestenyl)formate was obtained.

3β-(5-Cholestenyl) chloroformate was also coupled with phenylalanine, glutamine, and the ethyl ester of β-alanine. However, no product was isolated when the chloroformate was treated with methionine, cysteine, glutamic acid, or serine under identical conditions. Further attempts to prepare these derivatives of cholesterol by either changing the solvent system, refluxing time, or concentration ratio were all unsuccessful.

The potassium salts of the phenylalanyl and glycyl carbamates of cholesterol were prepared by dissolving the carbamates in *n*-butanol and adding, in portions, a 40% solution of potassium 2-ethylhexanoate in *n*-butanol until no further precipita-

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TABLE I.—CHLOROFORMATE AND AMINO ACID DERIVATIVES OF SELECTED STEROIDS

Compd.	M.p., °C[a]	Yield, %	Formula	Analyses, %[b] Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
Cholesterol-GC[c]	220–222	95	$C_{30}H_{49}NO_4$	73.92	74.16	10.12	9.67
Cholesterol-PC[c]	164–166	60	$C_{37}H_{55}NO_4 \cdot H_2O$	74.59	75.15	9.64	9.65
Cholesterol-GlC[c]	138–140	60	$C_{33}H_{54}N_2O_5$	70.92	70.36	9.76	9.81
Cholesterol-GC, K salt	240–242	85	$C_{30}H_{48}NO_4K$	68.53	67.81	9.19	9.75
Cholesterol-PC, K salt	195–197	70	$C_{37}H_{54}NO_4K$	72.15	71.83	8.83	9.02
Cholesterol-GlC, K salt	212–215	80	$C_{33}H_{53}N_2O_5K$	66.43	65.98	8.95	8.61
Cholesterol-AC[c]	100–102	50	$C_{32}H_{55}NO_4$	74.81	75.56	10.46	10.22
9α-Fluorohydrocortisone-GC	165–166	80	$C_{24}H_{32}FNO_8$	59.87	60.70	6.69	7.32
9α-Fluorohydrocortisone-PC	165–167	75	$C_{31}H_{38}FNO_8$	65.13	65.21	6.70	7.41
9α-Fluorohydrocortisone-GlC	170–172	60	$C_{27}H_{43}FNO_9$	58.65	60.01	6.75	7.45
Fluandrenalone chloroformate	175–177	90	$C_{25}H_{32}ClFO_7$	60.20	60.85	6.27	6.62
Fluandrenalone-GC	164–166	40	$C_{27}H_{36}FNO_9$	60.10	60.14	7.15	6.97
Fluandrenalone-GC, K salt	>300	75	$C_{27}H_{35}FNO_9K$	56.33	55.93	6.13	6.41
2-Methyl-dihydrotestosterone chloroformate	153–156	60	$C_{21}H_{31}ClO_3$	68.85	69.50	8.54	8.15
2-Methyldihydrotestosterone-GC	209–211	25	$C_{23}H_{35}NO_5$	68.20	68.69	8.69	8.44
2-Methyldihydrotestosterone-GC, K salt	223–226[d]	80	$C_{23}H_{34}NO_5K$	62.67	62.45	7.72	8.00

[a] Melting points are uncorrected. [b] Analyses by Weiler and Strauss, Oxford, England. [c] GC is *N*-glycyl carbamate, PC is *N*-phenylalanyl carbamate, GlC is *N*-glutamine carbamate, AC is *N*-(β-alanyl ethyl ester) carbamate. [d] With decomposition.

tion was noticed. The potassium salt of the glycyl carbamate was hygroscopic.

The product obtained after the treatment of 9α-fluorohydrocortisone with phosgene, containing only one chloroformyl group, has been previously described (1). Similarly, it was found that only a monochloroformate of fluandrenalone (6α-fluoro-16α,17α-isopropylidenedioxy-Δ⁴-pregnen-11β,21-dihydroxy-3,20-dione) could be prepared. Treatment of 9α-fluorohydrocortisone and fluandrenalone with glycine, phenylalanine, and glucamine gave the desired carbamates.

An attempt was also made to prepare various amino acid derivatives of 2α-methylandrostan-3-one-17β-ol(2-methyl-dihydrotestosterone). The chloroformate was obtained in a 70% yield, but only the glycine derivative was obtained in a pure form. The incompleteness of the reaction and the difficulty in finding a good purification system affected the preparation of the other amino acid derivatives. The physical properties, analyses, and yields of the various chloroformate and amino acid derivatives are given in Table I.

Infrared Data.—The significant changes which could be observed in the infrared spectra between 2.5 and 7.0 μ of the amino acid derivatives can be attributed to the particular product obtained.

The carbonyl band of the chloroformyl radical exhibits a maximum between 5.62 and 5.63 μ for all of the steroids. The carbonyl band for the substituted carbamates exhibits a maximum between 5.80 and 5.95 μ. The amino acid derivatives also show a new band between 6.5 and 6.6 μ which is attributed to the amide II band of secondary amides or secondary carbamates. The carboxyl group of the amino acids appears as a maximum at 5.70 to 5.82. When the potassium salt was prepared, the carboxyl band at 5.70 to 5.82 μ shifts to 6.20 to 6.30 μ (8). The band at 5.94 to 6.05 μ in some steroids is attributed to the 3-keto function.

Thus, the presence of the characteristic carbamate band, the carboxyl band, the ability of the amino acid compounds to form pure potassium salts, and the preparation of a carbamate with the ethyl ester of β-alanine furnishes evidence that the derivatives are carbamates and not carbonates. The characteristic maxima of the compounds are listed in Table II.

Solubility Studies.—Since the primary objective of this project was a further attempt to prepare a series of steroid derivatives which would have an increased solubility in water over the parent steroids, a solubility study was undertaken. Utilizing the gravimetric procedure previously described (1), it was found that the amino acid moiety had very little effect on the water solubility of the parent steroid. However, when the potassium salt of the glycyl derivative was tested, it increased the solubility of cholesterol 600 times, fluandrenalone 100 times, and 2α-methyl-dihydrotestosterone 200 times. The potassium salt of the glutamine derivative was as effective in increasing the solubility of cholesterol, but the potassium phenylalanyl derivative was only one-half as effective.

Preliminary Pharmacological Results.—At the present time, the potassium salt of the glycyl derivatives of 2-methyl-dihydrotestosterone and fluandrenalone have been tested in the pharmacology laboratories of Eli Lilly and Co.

The fluandrenalone-glycyl carbamate when administered subcutaneously to mice or rats at a dose level of 0.25 to 1.0 mg./Kg. was inactive in either anti-inflammatory or antiestrogenic studies. The 2-methyl-dihydrotestosterone compound, when tested for myotrophic-androgenic activity by subcutaneous injection in rats, was about one-third to one-fourth as active as Dromostanolone propionate (myotrophic-androgenic ratio of 1.58). At present, the studies of the same compounds given by oral administration have not been completed.

EXPERIMENTAL

Where a number of compounds were synthesized by a similar method, a typical preparation is given.

6α - Fluoro - 16α,17α - isopropylidenedioxy - Δ⁴-pregnen-11β-hydroxy-3,20-dione-21-chloroformate.—A solution of 2 Gm. (0.0044 mole) of fluandrenalone (Cordran, Eli Lilly and Co.) in 50 ml. of tetrahydrofuran was saturated with phosgene. The solution was allowed to stand for 3 hours, and the

TABLE II.—INFRARED ABSORPTION CHARACTERISTICS

Compd.	Absorption Maxima, μ[a]						
Cholesterol	2.80	...	...	...	...	...	...
" chloroformate	...	5.62	...	...	...	...	...
" GC[b]	2.90	...	5.70	5.95	...	...	6.35
" GC, K salt	2.95	...	...	5.90	...	6.20	6.55
" PC[b]	2.80	...	5.73	5.89	...	...	6.55
" GlC[b]	2.85	...	5.73	5.89	6.00	6.30	6.45
" GlC, K salt	2.80	...	...	5.89	6.00	6.20	6.50
" AC[b]	2.80	...	5.71, 5.78	5.82	...	...	6.60
9α-Fluorohydrocortisone	2.90	...	...	5.80	6.01	...	...
" chloroformate	2.85	5.60	...	5.90	6.10	...	...
" GC	2.80	...	5.79	...	6.00	...	6.50
" PC	2.85	...	5.75	...	6.05	...	6.60
" GlC	2.86	...	5.77	...	6.00	...	6.50
Fluandrenalone	2.83	...	...	5.83	5.94	...	...
" chloroformate	2.84	5.66	...	5.78	6.00	...	6.60
" GC	2.83	...	5.75	5.80	6.00	...	6.50
" GC, K salt	2.85	...	...	5.80	6.00	6.25	6.50
2α-Methyldihydrotestosterone	2.82	...	...	5.80	5.95	...	...
" chloroformate	...	5.62	...	5.80	6.00	...	...
" GC, K salt	2.82	...	5.75	5.80	5.95	...	6.50
" GC, K salt	2.88	...	...	5.80	5.92	6.25	...

[a] All determinations were made on a Perkin-Elmer model 137 Infracord spectrophotometer. Samples were mounted as Nujol mulls. [b] GC is *N*-glycyl carbamate, PC is *N*-phenylalanyl carbamate, GlC is *N*-glutamine carbamate, and AC is *N*-(β-alanyl ethyl ester) carbamate.

solvent was removed *in vacuo*. The residue was dissolved in hot acetone, the solution was treated with charcoal, and was filtered. The solvent was allowed to evaporate spontaneously; the product which remained melted at 175–177°. The compound darkened on exposure to moisture and light.

21-(6α-Fluoro-16α,17α-isopropylidenedioxy-Δ^4-pregnen-11β-hydroxy-3,20-dione)-N-(carboxymethyl)carbamate Potassium Salt.—To 1.5 Gm. (0.003 mole) of 6α-fluoro-16α,17α-isopropylidenedioxy-Δ^4-pregnen-11β-hydroxy-3,20-dione-21-chloroformate in 30 ml. of acetone was added 0.75 Gm. (0.01 mole) glycine (Merck and Co., Inc.) in 30 ml. of water. The mixture was refluxed for 4 hours, filtered hot, and the resulting filtrate was concentrated by spontaneous evaporation. The resulting precipitate was collected on a filter. Purification of the product was accomplished by treating an acetone solution of the compound with charcoal and recrystallization from ethanol. The tan product, after drying, melted at 164–166°.

The potassium salt was prepared by dissolving 0.35 Gm. of the compound in 5 ml. *n*-butanol and adding 0.5 ml. of a 40% solution of potassium 2-ethylhexanoate in *n*-butanol. The resulting precipitate was collected on a filter and dried. An 80% yield of the water-soluble product, m.p. 212–215°, was obtained.

17β-(2α-Methylandrostan-3-one)N-(carboxymethyl)carbamate Potassium Salt.—To 1.84 Gm. (0.005 mole) of 2α-methylandrostan-3-one-17β-yl-chloroformate (obtained in the usual manner from 2α-methyl-5A-dihydrotestosterone, Eli Lilly and Co.) in 50 ml. of dioxane was added 0.75 Gm. (0.01 mole) glycine in 25 ml. of water. The mixture was refluxed for 2 hours and was allowed to stand overnight. It was again refluxed for 2 hours, cooled, and the solvent evaporated *in vacuo*. The resulting product was recrystallized from acetone. A 25% yield of white platelets, m.p. 205–208°, was obtained.

The potassium salt, prepared in the previously described manner, was obtained in an 80% yield and melted with decomposition at 221–224°.

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