tion from aqueous methanol gave a product (426 mg.) which melted at 52–55°, partially solidified, and then melted at 92–94°.

Anal. Calcd. for $C_{23}H_{34}O_2$: C, 80.67; H, 10.00. Found: C, 80.61; H, 10.07.

17-Butyl-19-nortestosterone.—The Birch reduction of 17-butylestradiol 3-methyl ether and the cleavage and rearrangement of the resulting 1,4-dihydro derivative was carried out by the procedure previously described for the 3-methyl ether of 17-ethylestradiol. The product from the reaction was purified by chromatography over silica gel (35 g.). Elution with 20% ethyl acetate in benzene followed by crystallization from aqueous methanol gave 118 mg. of 17-butyl-19-nortestosterone which melted at 126–127°, λ_{max} 240.5 mμ, log E 4.23. The infrared spectrum (KBr disk) shows bands at 2.82, 6.03, 6.22, 7.92, 8.21, 9.81, 10.39 and 11.31 μ.

Anal. Calcd. for $C_{22}H_{34}O_2$: C, 79.95; H, 10.37. Found: C, 79.66; H, 10.53.

CHICAGO 80, ILLINOIS

[CONTRIBUTION OF THE RESEARCH LABORATORIES, THE UPJOHN CO.]

The Separation of Stigmasterol from Soybean Sterols[1]

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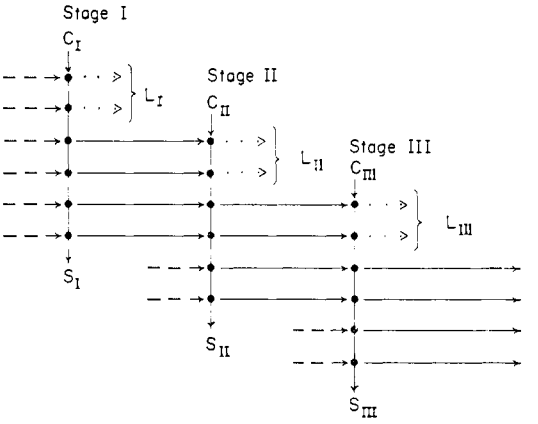
Several new esters of stigmasterol and a new process for the isolation of stigmasterol of about 88% or better purity from soybean sterols *via* the α-naphthylcarbamates are described.

Stigmasterol, one of the most abundant raw materials for the synthesis of steroid hormones,[2] was separated from soybean sterols[3] in 1911 by Matthes and Dahle.[5] The separation method was applied earlier by Windaus and Hauth[6] who isolated stigmasterol from the sterols of calabar beans. The Windaus–Hauth process has been the classical method for the isolation of stigmasterol and a method for its estimation. This process was shown to be inefficient by Neu and Ehrbächer[7] and by Johnson, Donia and Ott.[8] Analysis of given lots of soybean sterols by both radioactive isotope dilution[9] and infrared assays[10] showed that about twice as much stigmasterol was present as was indicated by the Windaus–Hauth separation. On these bases crude soybean sterols were believed to contain more stigmasterol than previously indicated. One objective of this study was to confirm by actual isolation the stigmasterol content indicated by the newer assay methods.

A study of several esters (Table I) of soybean sterols showed that stigmasterol α-naphthylcarbamate could be effectively separated from the other soybean steryl α-naphthylcarbamates. Other carbamate esters studied are less efficient and the carboxylic acid esters have little utility for the separation of stigmasterol.

The most efficient process for the separation of the soy steryl α-naphthylcarbamates was a discontinuous countercurrent leaching process described in Fig. 1. The important factors affecting the leaching were the volume of solvent per pass[11] and the number of passes required to dissolve the sitosteryl α-naphthylcarbamates. Other variables, particle size, temperature, rate and duration of stirring had little effect within reasonable limits.

Figure 1.
Discontinuous Countercurrent Leaching Process



Legend:
- • A pass
- path of solids is down
- path of liquors is left to right
- → fresh chlorobenzene
- → liquors reused
- C = charge of crude soy steryl α-naphthylcarbamates
- S = solid stigmasteryl α-naphthylcarbamate
- L = liquors containing sitosteryl α-naphthylcarbamates

Separations of the soy steryl α-naphthylcarbamates as described in Fig. 1 gave, after hydrolysis, stigmasterol of 88 to 93% purity by infrared analysis in 90 to 96% of the theoretical amount indicated by the infrared analysis of the starting sterols.[12] The yields and purities correspond to a recovery of about 85% of the stigmasterol. The product thus isolated was identical in m.p., optical

(1) Presented before the Division of Organic Chemistry at the 128th Meeting of the American Chemical Society, Minneapolis, Minn., September 11–16, 1955.

(2) F. W. Heyl and M. E. Herr, THIS JOURNAL, **72**, 2617 (1950).

(3) Soybean sterols as obtained from the unsaponifiable fraction of soybean oil generally contain 12–25% stigmasterol; the remainder is largely various sitosterols.[4]

(4) K. S. Markley, "Soybean and Soybean Products," Vol. II, Interscience Publishers, Inc., New York, N. Y., 1951, p. 837.

(5) H. Matthes and A. Dahle, *Arch. Pharm.*, **249**, 436 (1911).

(6) A. Windaus and A. Hauth, *Ber.*, **39**, 4378 (1906).

(7) R. Neu and P. Ehrbächer, *Arch. Pharm.*, **283**, 227 (1950).

(8) B. A. Johnson, R. A. Donia and A. C. Ott, unpublished work from the Upjohn Co.

(9) R. A. Donia, N. A. Drake and A. C. Ott, *Anal. Chem.*, in press.

(10) J. L. Johnson and A. O. Jensen, *Anal. Chem.*, to be published.

(11) In these studies a leaching operation (or pass) consisted of stirring a mixture of the solid carbamate with a solvent and separating the phases by filtration. The stigmasteryl α-naphthylcarbamate remained in the undissolved portion.

(12) The soy sterols used in these studies contained 17 to 24% stigmasterol by infrared assay.

TABLE I

DERIVATIVES OF STIGMASTEROL

Derivative[a]	M.p. (Kofler), °C.	$[\alpha]D$ ($CHCl_3$)	Formula	Analyses, % Calcd. C	Calcd. H	Found C	Found H	Solvents[d] for recrystln. Stig. deriv.	Soy S. deriv.	Ref. to exptl.
Acetoacetate[a]	121–124	−45°	$C_{33}H_{52}O_3$	79.78	10.56	79.72	10.36	*i*P–M	*i*P–M	I
itrobenzoate[b]	206–207	−15	$C_{36}H_{51}NO_4$	76.96	9.17	77.11	9.17	E	E	I
m-Bromobenzoate	160–161.5	−19	$C_{36}H_{51}BrO_2$	72.58	8.63	72.89	8.50	E	A	I
3,5-Dinitrobenzoate	234–235	−21	$C_{36}H_{50}N_2O_6$	71.25	8.30	71.12	8.04	E	E	I
α-Naphthoate	178–179	−29	$C_{40}H_{54}O_2$	84.75	9.60	84.85	9.50	E	A	I
Cyclohexylcaproate	103–106	−38	$C_{41}H_{68}O_2$	83.04	11.56	83.28	11.40	A	..	I
β-Phenylpropionate	126.5–127	−37	$C_{38}H_{56}O_2$	83.76	10.37	84.39	10.18	A–3A alc.	..	I
Carbamate	225–229	−48	$C_{30}H_{49}NO_2$	79.06	10.84	79.07	11.12	E	2-Butanone	IIIB
Methylcarbamate	205–210	−45	$C_{31}H_{51}NO_2$	79.26	10.94	79.51	10.64	E	Benzene	IIIA
n-Butylcarbamate	139–141	−38	$C_{34}H_{57}NO_2$	79.78	11.23	80.02	11.36	A–M[e]	A	IIIB
Diethylcarbamate	146–148.5	−34	$C_{34}H_{57}NO_2$	79.78	11.23	80.03	11.09	A[e]	A	IIIB
Octadecylcarbamate	104–105	−27	$C_{48}H_{85}NO_2$	81.40	12.10	81.53	11.89	E	..	IIIA
Phenylcarbamate	194.5–196.5	−38	$C_{36}H_{55}NO_2$	81.30	10.04	81.70	10.13	C[e]	E–C	IIIA
Phenylthiocarbamate	180–181.5	−26	$C_{36}H_{55}NOS$	78.92	9.75	79.08	9.75	Toluene	SSC–C	IIIA
p-Nitrophenylcarbamate	228–230	−34	$C_{36}H_{54}N_2O_4$	74.96	9.09	74.94	9.23	E[e]	E	IIIA
o-Tolylcarbamate	166–168.5	−35	$C_{37}H_{55}NO_2$	81.41	10.15	81.75	10.24	A	A	IIIA
p-Tolylcarbamate	194–196.5	−36	$C_{37}H_{55}NO_2$	81.41	10.15	81.71	10.41	SSC	C	IIIA
o-Biphenylcarbamate	194–196	−18	$C_{42}H_{57}NO_2$	82.98	9.45	83.18	9.30	C	C	IIIB
Dehydroabietylcarbamate	154–159	+1	$C_{50}H_{77}NO_2$	82.93	10.72	83.65	10.84	A	E–3A alc.	IIIB
Carbazolylformate	213–215	−26	$C_{42}H_{55}NO_2$	83.26	9.15	83.53	9.14	E	E	IIIB
Chloroformate	115–120	−38	$C_{30}H_{47}ClO_2$	75.83	9.97	76.27	9.96	Dry A	Dry A	II
α-Naphthylcarbamate	213–217	−22	$C_{40}H_{55}NO_2$	82.56	9.53	82.21	9.38	C–CH_2Cl_2	C	IIIA, IIIB

[a] Reference 14. [b] J. C. E. Simpson and M. E. Williams, *J. Chem. Soc.*, 733 (1937). [c] Chromatographed through Florisil before crystallization. [d] E = ethyl acetate; A = acetone; M = methanol; C = cyclohexane; SSC = Skellysolve C, *i*P = isopropyl ether. [e] The infrared spectra are consistent with the proposed structures.

rotation and infrared absorption spectrum to authentic stigmasterol.

Stigmasterol purity was determined throughout by infrared assay. Use of these data to confirm the validity of the infrared and isotope dilution required not only that the indicated amount of stigmasterol be isolated but that it be identified by an independent method. Therefore, the product was oxidized in high yield by the Oppenauer procedure to stigmastadienone. The presence of the double bond was demonstrated by ozonolysis of the dienone in 84% yield to 3-ketobisnor-4-cholenaldehyde. The identity of the stigmastadienone and 3-ketobisnor-4-cholenaldehyde with authentic samples proved the identity of the isolated stigmasterol and confirmed the accuracy of the infrared and isotope dilution assays.

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Experimental[13]

Stigmasterol and Soy Sterylcarboxylic Acid Esters (Expt. I).—The stigmasterol or soy sterols were dissolved in methylene chloride and the solution dried by distilling a small portion of the solvent. To the dried solution was added 1.2 to 1.5 molecular equivalents of the acid chloride and an excess of pyridine. After being warmed 2 to 3 hr. or standing overnight, the solution was washed with dilute acid, dilute alkali and water, dried over magnesium sulfate, filtered and concentrated to dryness. The stigmasterol derivatives were crystallized and the soy steryl esters fractionally crystallized from the solvents indicated in Table I.

Stigmasteryl and soy steryl acetoacetates were prepared according to Bader, Cummings and Vogel.[14]

Stigmasteryl and Soy Steryl Chloroformates (Expt. II).—Twenty grams (0.0486 mole) of soy sterols containing 18% stigmasterol by infrared assay was dissolved in 200 ml. of boiling methylene chloride, slurried with Darco and filtered through a bed of Celite. The filtrate was cooled to 25–30° and kept at that temperature range while phosgene was passed into the solution for 1 hr. During this time the sterols precipitated and redissolved. The methylene chloride was removed by distillation and the residue of soy steryl chloroformates freed of traces of solvent and phosgene under reduced pressure. The product was used without purification to prepare the various carbamates. Stigmasteryl chloroformate was prepared in a similar manner.

Stigmasterol and Soy Steryl Carbamate Esters (Expt. III). A. From Isocyanates.—Stigmasterol or soy sterols were dissolved in boiling cyclohexane and a small amount of the solvent distilled to remove any trace of water. A few drops of pyridine and 1.2 to 1.5 molecular equivalents of the isocyanate were added and the solution heated under reflux for about 5 hr. The desired carbamate crystallized from the reaction mixture upon cooling. The stigmasteryl carbamates were recrystallized, and the soy steryl carbamates were fractionally crystallized from the solvents indicated in Table I.

B. From Chloroformates and an Amine.—Stigmasteryl or soy steryl chloroformates were dissolved in benzene or methylene chloride, and about 2.5 molecular equivalents of the dry amine was added. After warming slightly to ensure complete reaction, the amine hydrochloride was removed by filtration, and the filtrate was washed with dilute acid and water, dried over magnesium sulfate, filtered and concentrated. The derivatives were crystallized from the solvents indicated in Table I. The methylcarbamates were prepared according to McKay and Vavasour.[15]

Soy Steryl α-Naphthylcarbamate (Expt. IV).—Soy sterols, 333 g. (0.805 mole), were melted in a 1-l. round-bottom flask under vacuum. This removed the air and water from the mixture. The vacuum was released, and to the melt at 150° were added 10 ml. of dry 2,4-lutidine and 138 g. (0.82 mole)

(13) The melting points were observed on a Kofler micro hot-stage checked against standard compounds. Infrared spectra were determined with a Perkin–Elmer model 21 spectrophotometer equipped with NaCl prisms on mulls of the compounds in Nujol (liquid petrolatum). αD's were determined at 22–26° in concentrations of 1–1.5 g. per 100 ml. in a 2-dm. tube. The infrared assay measured the stigmasterol in chloroform solution in terms of absorption by the *trans* double bond in the side chain at 975 cm.$^{-1}$ (ref. 10). Care must be exercised in handling α-naphthyl isocyanate and α-naphthylamine because all known commercial samples of α-naphthylamine contain some of the highly carcinogenic β-naphthylamine.

(14) A. R. Bader, L. O. Cummings and H. A. Vogel, THIS JOURNAL, **73**, 4195 (1951).

(15) A. F. McKay and G. R. Vavasour, *Can. J. Chem.*, **31**, 688 (1953).

of α-naphthyl isocyanate. The melt was stirred slowly for 0.5 hr. during which time the temperature rose to 185°. The melt was poured into a large tray to crystallize. After cooling the product was broken from the tray and ground. The yield was 455 g., 98%.

Hydrolysis of Steryl Carbamates (Expt. V). A. Potassium Hydroxide–Methyl Cellosolve.—Potassium hydroxide, 0.35 g. (0.005 mole) was dissolved in 1.5 ml. of water, and 20 ml. of Methyl Cellosolve (glycol monomethyl ether), and 0.001 mole of the steryl carbamate were added. The resulting slurry was heated under reflux for 45 minutes, at which time the solution was clear except for a small amount of precipitated potassium carbonate. While the solution was still hot, 5 ml. of water was added dropwise with stirring. A precipitate of the free sterol formed during this addition. The slurry was cooled to room temperature, an additional 5 ml. of water was added, and the precipitate was filtered, washed very thoroughly with five portions of water and dried. In some cases one water wash was replaced with a wash of dilute (2 *N*) hydrochloric acid to facilitate removal of the amine and carbonate formed in the hydrolysis. The yield of sterol was 0.40 to 0.42 g. (95 to 100%) when the carbamate was not contaminated with non-sterylcarbamate impurities such as the substituted ureas.

The *n*-butyl carbamates failed to hydrolyze satisfactorily in Methyl Cellosolve (b.p. 122°) but were hydrolyzed in 2 hr. in boiling propylene glycol containing a small amount of water and the usual amount of potassium hydroxide.

The diethyl carbamates failed to undergo hydrolysis in 4.5 hr. in boiling ethylene glycol containing water and potassium hydroxide.[16]

B. **Toluene–Methyl Cellosolve.**—Sodium hydroxide, 2.4 g. (0.06 mole), was dissolved in 2.4 ml. of water and 80 ml. of Methyl Cellosolve, 20 ml. of toluene and 11.64 g. (0.02 mole) of stigmasteryl α-naphthylcarbamate added successively. The mixture was boiled under reflux for 3 hr. and water and toluene were added. The toluene layer was separated while warm and washed with 2 *N* hydrochloric acid and water. The toluene phase was concentrated to dryness to yield 8.0 g. (95%) of stigmasterol, $[\alpha]_D$ −49° ($CHCl_3$), purity (infrared assay), 92.2%.

Solubility of Stigmasteryl α-Naphthylcarbamate in the Presence of Varying Amounts of Sitosteryl α-Naphthylcarbamates.—Soy steryl α-naphthylcarbamates (12.5 g.) containing 21% of the stigmasterol derivative were slurried for 0.5-hr. periods with six 25-ml. portions of chlorobenzene. After each leaching the liquor was separated by filtration and concentrated to dryness. Each of the six liquors was hydrolyzed to the free sterol and assayed for stigmasterol content (Table II). The final insoluble residue, 1.60 g., was 88% stigmasteryl α-naphthylcarbamate.

Separation of Stigmasterol from Soy Sterols.—Soy sterols containing 22.7% stigmasterol by infrared assay were converted to the α-naphthylcarbamate derivatives by a method IV. Following the diagram in Fig. 1 five stages with a 117-g. charge per stage were processed using 234 ml. of chlorobenzene per pass and 6 passes per stage. The first two liquors of each stage were combined and hydrolyzed to give a mixture of sitosterols containing less than 2% stigmasterol. The insoluble residue from each stage was hydrolyzed (method VA) to yield stigmasterol. Stigmasterol thus isolated had a m.p. of 167–170° and $[\alpha]_D$ −49° ($CHCl_3$). Thus calculating from averages of the "steady" stages 2–5 in Table III, from 100 g. of soy sterols containing 22.7% stigmasterol, there was obtained 21.9 g. of product which was 88.6% stigmasterol.

TABLE II

SOLUBILITY OF STIGMASTERYL α-NAPHTHYLCARBAMATE DURING THE LEACHING OF SOY STERYL α-NAPHTHYLCARBAMATES WITH CHLOROBENZENE

Pass	Extracted solids g.	Extracted solids % stig.	Solubility of stig. carb., g./100 ml.
1	4.63	0–2	0 to 0.37
2	3.34	5	0.67
3	1.27	14	.71
4	0.72	24	.69
5	.40	46	.74
6	.32	68	.87

TABLE III

STIGMASTEROL FROM SOY STEROLS

Stage	Stig. carb. (S), g.	Hydrolysis yield, %	Stigmasterol Purity	Stigmasterol Recovery,[a] %
1	18.2	95.6	87.7	57.2
2	26.3	96.1	88.4	84.3
3	26.5	97.4	88.1	85.4
4	26.3	97.3	87.1	84.2
5	26.6	97.4	90.7	88.4
Average of stages 2–5	26.4	97.1	88.6	85.6

[a] Corrected for purity.

3-Ketobisnor-4-cholenaldehyde.[17]—The stigmasterol was oxidized by the Oppenauer[18] method to stigmastadienone, 79.7% yield, m.p. 124–126°, $[\alpha]_D$ +57° ($CHCl_3$), ϵ_{242} 15,300. The stigmastadienone was cleaved by ozonolysis[2] to 3-ketobisnor-4-cholenaldehyde, 84.7% yield, m.p. 160–167°, $[\alpha]_D$ +83° ($CHCl_3$). The infrared spectra of both the stigmastadienone and the 3-ketobisnor-4-cholenaldehyde were identical to those of materials prepared from stigmasterol isolated by the Windaus–Hauth process.

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(16) Difficultly hydrolyzable carbamates can be cleaved with $LiAlH_4$; R. L. Dannley, M. Lukin and J. Shapiro, *J. Org. Chem.*, **20**, 92 (1955).

(17) The Oppenauer oxidation was done by Y. F. Shealy and the ozonolysis by G. Slomp.

(18) R. J. Oppenauer, *Rec. trav. chim.*, **56**, 137 (1937).