LITERATURE CITED

1. K. T. Wang and I. S. Y. Wang, J. Chromatogr., 27, 318-320 (1967).
2. J. P. Brown and R. N. Perham, Eur. J. Biochem., 39, 69-73 (1973).
3. S. R. Burzynski, Anal. Biochem., 65, 93-99 (1975).
4. M. H. Joseph and J. Halliday, Anal. Biochem., 64, 389-402 (1975).
5. K. D. Kulbe and Y. M. Nogueira-Hattesohl, Anal. Biochem., 63, 624-629 (1975).
6. H. H. Lon, I. K. Ho, T. M. Cho, et al., J. Chromatogr., 76, 505-508 (1973).
7. H. H. Lon, I. K. Ho, T. M. Cho, et al., J. Chromatogr., 68, 289-293 (1972).
8. B. Wortmann, W. Wortmann, and J. C. Touchstone, J. Chromatogr., 70, 199-201 (1972).
9. Y. T. Huang, and K. T. Wang, J. Chromatogr., 31, 587-589 (1967).
10. K. T. Wang and P. H. Wu, J. Chromatogr., 37, 353-354 (1968).
11. D. Radulović, L. Blagojević, and D. Livanov-Stakić, Acta Pharm. Jugosl., 24, 173-176 (1974).
12. L. I. Brutko, V. E. Chichiro, D. Z. Yaskina, et al., Collection of the Scientific Transactions of the Central Experimental Scientific Research Institute [in Russian], Vol. 10 (1970), pp. 95-100.

IDENTIFICATION OF CERTAIN COMPONENTS OF WOOL FAT

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The neutral fraction obtained by the saponification of lanolin (the wool fat alcohols—WFA) is widely used in the pharmaceutical industry and cosmetic practice [1, 2]. It is known that the WFA contains several aliphatic alcohols, cholesterol [3], and many other physiologically active compounds. The technology of the preparation of WFA has been worked out [3]. Nevertheless, data on the chemical composition of WFA are rather incomplete. The composition of WFA may vary considerably depending on their origin. This makes it difficult to identify them.

In Kirghizia, lanolin is prepared in factories for the primary processing of wool by washing it with sodium carbonate solution in the presence of soap followed by the separation of the fat from the aqueous phase. Thus, a considerable part of lanolin (up to 40-50%) remains in the waste waters. Therefore, extraction of lanolin from the waste waters is of practical interest, since it makes it possible to increase the yield of lanolin and free the waters from fat.

We therefore extracted the waste waters by trichloroethylene, which was very effective. The low toxicity of trichloroethylene, its extremely low solubility in water, as well as its high specific weight make it possible to separate the liquid phases; thus the aqueous phase contains almost no organic residues.

It was interesting to study the composition of WFA obtained from lanolin isolated by extraction, and to identify some of the components. Wool fat alcohols obtained by the method already described [3] gave a positive Liebermann—Burkhardt test for sterols. However, in several cases this test gives much too high results. We therefore determined the content of sterols by the digitonin method [4]. According to this method, WFA contains 27% of sterols, which is considerably less than reported previously [3]. After the decomposition of digitonin by heating it with dimethyl sulfoxide by the method given in [5], followed by extraction with hexane, free sterol, mp 148-149°C (from alcohol), was isolated, and was found to be completely identical with cholesterol (comparison of the behavior on TLC, as well as of the data of IR and mass spectra with the corresponding data for an authentic sample).

The WFA residue which does not give a precipitate with digitonin was subjected to often repeated thin-layer chromatography on silica gel. Thus, five chromatographically individual fractions were obtained (see Table 1).

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TABLE 1. Chromatographic Separation on Silica Gel of the Neutral Fraction with No Sterols*

Fraction	R_f (chloroform)	Weight, kg	Melting point, °C	Note
№ 1	0,95	40	Oil	Mixture of C_{17}-C_{30} hydrocarbons
№ 2	0,70	5	"	4-Methyl-2,4-cholestadiene
№ 3	0,40	20	69—71	Lignoceric alcohol
№ 4	0,20	6	68—69	Behenic alcohol
№ 5	0,10	7	Oil	Cetyl alcohol

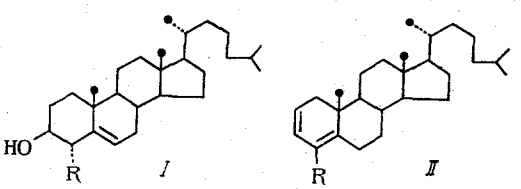
*Weight of neutral fraction before separation of 150 mg.

According to the IR spectrum, the slightly polar fraction No. 1 (R_f 0.95) does not contain alcohols (absence of absorption in the 3300-3600 cm^{-1}). According to mass spectroscopic data, this fraction is a mixture of C_{17}-C_{30} hydrocarbons with a normal structure. Peaks of the corresponding molecular ions with m/e 422, 408, 394, 380, 366, 352, 338, 324, 310, 296, 282, 254, 240 were detected, as well as peaks with lower intensity than that of the fragments with the general formula $C_nH_{2n+1}^+$ with m/e 407, 393, 379, 365, 351, 337, 323, 309, 281, 267, 253, 239, 225, 211, 197, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57, and 43.

Thus, WFA contains triaconsane, nonacosane, octacosane, heptacosane, hexacosane, pentacosane, tetracosane, tricosane, docosane, heneicosane, eicosane, nonadecane, octadecane, and heptadecane.

Fraction No. 2, according to the data of the IR spectrum, is also a hydrocarbon. The presence in the spectrum of a broad absorption band with ν_{max} = 1650-1605 cm^{-1} indicates the presence of double bonds. This was confirmed by a positive test with tetranitromethane (yellow coloring). According to mass spectroscopic data, this hydrocarbon has an empirical formula of $C_{28}H_{46}$ [a peak of the molecular ion with m/e 382, and peaks of fragments with m/e 367 (M^+—C_3H_7), 389 (M^+—C_6H_{13}) and 269 (M^+—C_8H_{17}) were observed]. In general, the mass spectrum of fraction No. 2, beginning from the m/e 382 peak, completely coincides with mass spectrum of 4α-methylcholesterol (I, R = Me) [6]. We can therefore assume that fraction No. 2 is a product of dehydration of this sterol.

According to the data of the UV spectrum, the compound contains a homoannular system of conjugated double bonds (λ_{max} 277 nm, ε 7500). Calculation according to increments [7] shows that in this case the compound is $\Delta^{2,4}$-diene (calculated value λ_{max} 278 nm); for a $\Delta^{3,5}$-diene, this value is 234 nm, and for a $\Delta^{5,7}$-diene, 282 nm. From these data, as well as from the character of fragmentation under electronic impact, we can assign the structure of 4-methyl-2,4-cholestadiene (II, R = Me) to the hydrocarbon from fraction No. 2.



This conclusion also agrees with the data of the PMR spectrum for II (R = Me) run with $CDCl_3$ (δ, ppm): singlet at 0.69 (C-18, 3H), singlet at 1.01 (C-19, 3H), doublet with a center at 0.87 (C-26 and C-27, 6H, J 6 Hz), doublet with a center at 0.94 (C-21, 3H, J 6.5 Hz), broadened singlet at 1.69 (C-28, 3H), and two multiplets with centers at 5.10 and 5.31 (olefinic H atoms, each with an intensity in 1 H).

Earlier [8], during the refining of oils, it was observed that cholesterol (I, R = H) can become dehydrated with the formation of 2,4-cholestadiene (II, R = H). It is possible that in our case (during saponification), a similar process takes place. In this case, 4-methyl-2,4-cholestadiene is an artifact, and 4ξ-methylcholesterol (II, R = Me) should be the native product.

The crystalline fraction No. 3 is an alcohol (presence of a broad band in the 3200-3550 cm^{-1} region in the IR spectrum, no band of the CO group). In the mass spectrum of this compound there is no peak of the molecular ion, but intense peaks of fragments with m/e 336

(M^+-H_2O), 308 (M^+-46), 280 ($M^+-18-2\cdot28$) were observed. Further fragmentation proceeds as in the aliphatic hydrocarbons with the formation of ions of the general formula $C_nH_{2n+1}^+$. The NMR spectrum of fraction No. 3 practically completely coincides with that of lignoceric alcohol. The identity of fraction No. 3 with the latter alcohol was also confirmed by oxidation to lignoceric acid, as well as by reduction of an authentic sample of lignoceric acid to lignoceric alcohol $C_{24}H_{49}OH$, and direct comparisons of these two compounds.

A crystalline alcohol, mp 68-69°C, was isolated from fraction No. 4, which differed from lignoceric alcohol. According to mass spectrum data [peaks of fragments with m/e 308 (M^+-18), 295 (M^+-CH_2OH), 280 (M^+-46) and peaks of fragments of the general formula $C_nH_{2n+1}^+$ were observed], this compound was behenic alcohol $C_{22}H_{45}OH$.

Finally, the more polar fraction No. 5, which could not be crystallized, according to the mass spectrum data [peaks of molecular ion with m/e 242, formed as the result of dehydration of the ion with m/e 224, peaks of ions with m/e 211 (M^+-CH_2OH), 196 (M^+-46), and several peaks of ions of the general formula $C_nH_{2n+1}^+$ were observed], is cetyl alcohol $C_{16}H_{33}OH$.

Thus, WFA, together with aliphatic alcohols and cholesterol, contain several aliphatic hydrocarbons, as well as, possibly, 4-methylcholesterol, a compound which plays an important role in the biosynthesis of steroids. We believe that because of the presence of considerable amounts of hydrocarbons, the generally accepted name for the neutral fraction of lanolin, namely "wool fat alcohols" is not entirely justified.

Investigations have shown that the neutral fraction of lanolin can serve as a suitable source for the preparation of lignoceric alcohol, which can also find technical application. Moreover, it would be highly interesting to isolate 4-methylcholesterol from this source also. Work in this direction is being continued.

EXPERIMENTAL

The IR, UV and NMR spectra were run on the UR-10, Specord UV-Vis and Varian XL-100 apparatus, respectively. Trimethylsiloxane was used as the internal standard when running the NMR spectra. The mass spectra were run on the MX-1309 apparatus at an ionizing voltage of 70 eV.

Saponification of Lanolin. A mixture of 10 g of lanolin, obtained by extraction from waste waters of the first purification of wool by means of trichloroethylene, 8 ml of 15% sodium hydroxide and 150 ml of methanol was boiled for 4 h. The solution was cooled and diluted with water, and the neutral components were extracted five times with petroleum ether (total volume 300 ml). The extract was washed with a saturated solution of common salt, and then with water, and then dried over anhydrous sodium sulfate. After the distillation of the solvent *in vacuo*, 3.6 g of neutral products were obtained, which were then used without additional treatment.

Isolation of Cholesterol. A 0.2 g portion of the oil obtained as described above was dissolved in 5 ml of ethanol, and a solution of 0.5 g of digitonin in 100 ml of 60% ethanol was added. The precipitate which separated was centrifugated and washed with ethanol, then extracted for 12 h in a Soxhlet apparatus by boiling ether, and dried *in vacuo* to constant weight. Thus, 120 mg of digitonide was obtained, which was then boiled for 20 min with dimethyl sulfoxide. The solution was cooled to room temperature, and the precipitate of sterols was extracted with hexane. The hexane extract was dried over sodium sulfate, the solution was evaporated *in vacuo*, and 49 mg of a crystalline compound, mp 148-149°C (from methanol) were obtained. This was completely identical with cholesterol.

The alcoholic solution, containing an excess of digitonin and a compound which did not form a complex with it, was evaporated to $^2/_3$ of the initial volume, then 50 ml of water added, and the mixture was extracted with hexane. After the usual treatment of the extract, 150 mg of an oil were obtained, which was then studied further.

Chromatographic Separation. The oil obtained in the preceding experiment, which did not contain sterols, was dissolved in a minimal amount of chloroform, and placed onto a plate with silica gel, fixed with 5% of gypsum. The chromatography was carried out in chloroform, and zones with R_f 0.95, 0.7, 0.4, 0.2, and 0.15 were isolated. Each of these zones was extracted by a mixture of chloroform and methanol (2:1). The extracts were evaporated *in vacuo*, and the chromatography was repeated not less than five times. The results are listed in Table 1.

Oxidation of Lignoceric Alcohol. A 15 mg portion of chromic anhydride in a mixture of 3 ml of methylene chloride and 3 ml of 80% acetic acid was added to 10 mg of lignoceric alcohol isolated from fraction No. 3, and the mixture was left to stand at room temperature for 3 h. The reaction mixture was diluted with water and extracted with chloroform. The extract was washed with water, and then with 5% sodium hydroxide solution. The alkaline solution was extracted thrice with chloroform, and then acidified with hydrochloric acid to a slightly acidic reaction to a Universal indicator. The extraction with chloroform yielded 6 ml of lignoceric acid, mp 86-87°C (from acetic acid), which was completely identical with an authentic sample.

4-Methyl-2,4-cholestadiene. Mass spectrum (20°C): m/e 382 (M^+, 100%), 367 (M^+-CH_3, 16%), 339 ($M^+-C_3H_7$, 18%), 297 ($M^+-C_6H_{13}$, 25%), 269 (M^+-113, 63%), 227 (41%). UV spectrum (in ethanol): λ_{max} 277 nm (ε 7500). NMR spectrum (in $CDCl_3$) is described in the theoretical part.

Lignoceric Alcohol, mp 69-71°C (from alcohol); mass spectrum (30°C): m/e 336 (M^+-H_2O), 323 (M^+-CH_2OH), 308 (M^+-46), 280 ($M^+-18-2\cdot28$), as well as peaks of fragments with m/e 309, 295, 281, 267, 253, 239, 225, 211, 197, 183, 169, 154, 141, 127, 113, 99, 85, 71, 57, 43. NMR Spectrum (in $CDCl_3$, δ, ppm): triplet with a center at 3.65 (CH_2OH, 2H), singlet at 1.26 (CH_2, 44H) and an asymmetric doublet with a center at 0.86 (CH_3, 3H, J 7 Hz).

Behenic Alcohol, mp 67-68°C (from alcohol). Mass spectrum (30°C): m/e 308 (M^+-H_2O), 295 (M^+-CH_2OH), 280 (M^+-26), 281, 267, 253, 239, 225, 211, 197, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57, 43.

Cetyl Alcohol, mass spectrum (30°C): m/e 242 (M^+), 224 (M^+-H_2O), 211 (M^+-CH_2OH), 196 (M^+-45), 197, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57, 43. IR spectrum (a film of the substance): 3350-3440 cm^{-1} (the OH group).

LITERATURE CITED

1. F. Neuwald, Öst. Apoth. Ztg., 10, 168 (1956).
2. E. N. Lazareva, E. Z. Kagan, and E. N. Druzhinina, Khim.-Farm. Zh., No. 5, 30 (1971).
3. M. A. Trunova, M. Kh. Gluzman, T. A. Timoshenko, et al., Khim.-Farm. Zh., No. 12, 27 (1973).
4. G. M. Yablonskaya, G. I. Segal', P. N. Fisher, et al., Mikrobiol. Prom., No. 8, 8 (1973).
5. C. H. Issidorides, I. Kitagawa, and E. Mosettig, J. Org. Chem., 27, 515 (1962).
6. R. A. Okerholm, P. I. Brecher, and H. H. Wotiz, Steroids, 12, 435 (1968).
7. R. B. Woodward, J. Am. Chem. Soc., 64, 72 (1942).
8. P. Kaufmann and Y. Hamza, Fette Seifen, Anstrichmittel, 72, 423 (1972).