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Synthetic Emulsifying Agents

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Various synthetic compounds containing both lipophilic and hydrophilic groups have been prepared and their emulsifying properties examined. Conjugates of various amino acids with stearylamine, n-$C_{18}H_{37}NHCOCH(R)NH_2$, have been found to have promising emulsifying properties. These contrast with peptides of the type $C_{17}H_{35}CONHCH(R)COOH$, which are, at the most, weak emulsifying agents.

The work described in this communication was directed toward synthesis of emulsifying agents suitable for use in preparation of fat emulsions for intravenous injection. We limited our attention to substances either structurally similar to products natural to the animal organism or composed of units related to natural products with the hope that they would be susceptible to normal metabolism.

Natural lecithin has been used for this purpose with some success,[3] but has the disadvantage that it is difficult to obtain pure and, being of natural origin, it may be contaminated with allergens. Synthetic lecithins are available by the elegant procedure of Baer,[4] but we hoped that simpler compounds that are more amenable to synthesis would have similar emulsifying properties and perhaps be more resistant to hydrolysis.[5] Phosphorylcholine esters were prepared from two long-chain alcohols, octadecanol and dihydrophytol, and from cholestanol by the general method of Baer.[4] The products, both intermediate and final, are extremely

$$ROH + Cl_2POC_6H_5 \longrightarrow ROP(Cl)OC_6H_5 \longrightarrow$$

$$ROP(OC_6H_5)OCH_2CH_2\overset{+}{N}(CH_3)_3\ Cl^- \longrightarrow$$

$$ROP(OH)OCH_2CH_2\overset{+}{N}(CH_3)_3OH^-$$

difficult to obtain in pure form, and since they were inferior to soya bean lecithin in emulsifying properties, this particular approach was not explored further.

In theory, the requirement for emulsifying properties in a given substance is proper balance of lipophilic and hydrophilic characteristics. Since one objective was a study of the effect of various structural modifications on surface-active properties, we were interested particularly in substances available by relatively simple synthetic procedures. In addition, since most surface-active agents containing ionic groups are hemolytic, substances were sought in which the hydrophilic property is associated with a neutral group. Hexose or related units seemed potentially suitable hydrophilic units, and several types of substances were prepared in which a long-chain alkyl group is attached through a peptide bond to a sugar unit. A peptide linkage seemed desirable since it is more stable to hydrolysis than an ester linkage and, in addition, has some hydrophilic properties.[6] One group of substances investigated was the N-alkylamides of the aldonic acids, arabonic acid and gluconic acid. The former [$CH_2OH(CHOH)_3CONHR$] were prepared by condensation of a primary amine, n-decylamine and higher homologs, with methyl arabonate; the latter [$CH_2OH(CHOH)_4CONHR$] were synthesized by condensation of the amine with gluconolactone. The arabonamides are only slightly soluble in water and have no emulsifying properties. The gluconamides are more soluble in water and have weak emulsifying properties, which are improved considerably by use of a coemulsifier such as cholesterol,[7] but the resultant emulsions break after a few days. Attempts were made to improve the water-solubility by extension of the sugar chain, but surprisingly N-stearoylglucoheptonamide is less soluble in water than N-stearoylgluconamide.

Another group of related substances investigated was the N-alkyl derivatives of glucuronamide (III) and of glucososaccharonamide (V). The former were prepared in excellent yield by condensation of various primary amines with the 1,2-isopropylidene derivative of glucofururonolactone (I).[8] The reaction occurs at room temperature or below; dioxane or tetrahydrofuran are both suitable solvents for the reaction. The protective group is readily removed by acid hydrolysis to give the N-alkylglucuronamides, formulated III in the pyranose form, although the furanose structure is not excluded. Glucuronamide itself can be prepared similarly by use of concentrated aqueous ammonia; the yield is superior to that of a previous method involving the reaction of gaseous ammonia with glucuronolactone (glucurone).[9] An alternate route to compounds of type III involves the reaction of primary amines with the β-methylglucoside of glucuronolactone (IV).[8] According to the experimental conditions two forms (V) are obtained. These differ in solubility relationships and in melting point (intermediate mixed melting points), but in other respects, optical rotation and infrared absorption, they are identical, and both are hydrolyzed by acid to the alkylamides III. The difference may be

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(3) F. J. Stare and R. P. Geyer, *Surgery, Gynecology and Obstetrics*, **92**, 246 (1951); E. M. Neptune, Jr., R. P. Geyer, I. M. Saslaw and F. J. Stare, *ibid.*, **92**, 365 (1951).

(4) E. Baer and M. Kates, THIS JOURNAL, **72**, 942 (1950).

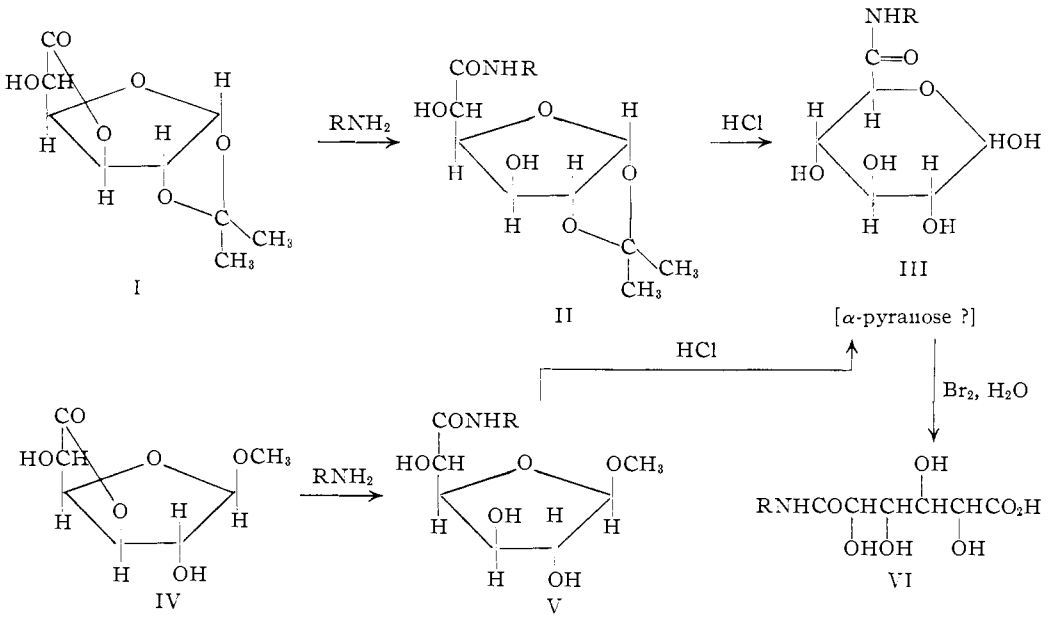
(5) Lecithins are fairly readily hydrolyzed to the toxic lysolecithins.

(6) E. Katchalski, *Advances in Protein Chemistry*, **5**, 123 (1951).

(7) J. H. Schulman and E. G. Cockbain, *Trans. Faraday Soc.*, **36**, 651 (1940).

(8) L. N. Owen, S. Peat and W. J. G. Jones, *J. Chem. Soc.*, 339 (1941).

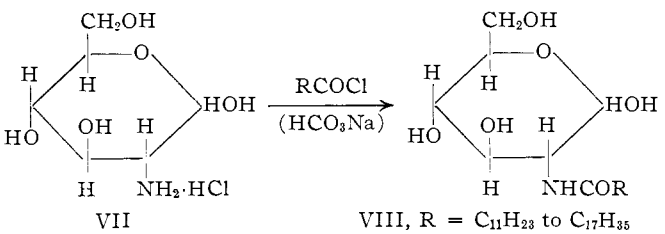
(9) H. L. Frush and H. S. Isbell, *J. Research Natl. Bur. Standards*, **41**, 609 (1948); U. S. Patent 2,504,984 (1950).



I II III IV V VI

due to polymorphism. The N-alkylglucosaccharonamides (VI) were prepared by bromine oxidation of the glucuronamides (III). None of the compounds exhibited appreciable solubility in water and none show emulsifying properties.

We also prepared a series of N-acylglucosamines (VIII) by the condensation of glucosamine hydrochloride (VII) with an acid chloride,[10] lauroyl–stearoyl. These hexose derivatives have even less solubility than the isomeric N-alkylglucuronamides (III); they have no emulsifying properties. That none of the various sugar derivatives described showed any appreciable solubility in water, even when the lipophilic substituent is relatively weak, may be because internally situated hydroxyl groups have less hydrophilic effect than terminal ones.



VII VIII, R = $C_{11}H_{23}$ to $C_{17}H_{35}$

Another group of substances investigated consisted of N-stearoylamino acids, $C_{17}H_{35}CONHCH(R)COOH$. These resemble the bile acid conjugates, important natural emulsifying agents, in that a lipophilic residue is linked through a peptide bond to an amino acid. In this series the nature of the amino acid residue has a pronounced effect on the solubility relationships of the peptide. Conjugates of stearic acid with taurine and with aspartic and glutamic acid are appreciably soluble in water, whereas those with neutral amino acids, *e.g.*, β-alanine and glycine, have very slight water solubility. The former conjugates have weak emulsifying properties. We also considered the possibility that peptides of α-aminostearic acid with simple amino acids might have emulsifying properties. This line was not investigated to any extent where it was found that the peptide of α-aminostearic acid and *dl*-alanine was relatively insoluble, even in hot water, and showed no surface-active properties.

We also prepared peptides of the type $n\text{-}C_{18}H_{37}NHCOCHR(NH_2)$, that is, conjugates of stearylamine with amino acids. These differ from the above peptides in the nature of the free end groups (amino rather than carboxyl) and also in that the lipophilic residue is linked to the nitrogen of the peptide bond rather than to the carbonyl group. The nature of the amino acid residue has an even more pronounced effect on the water solubility than in the peptides described above. Thus the conjugate of stearylamine with L-leucine is practically insoluble in water, whereas that with L-alanine is very soluble. Steric factors are also involved, since the conjugate with DL-alanine is less soluble than the conjugate with L-alanine. We also investigated conjugates with readily available acids such as crotonic, methylacrylic, lactic and glycolic. None compared with the alanine conjugate in water solubility. The size of the alkyl lipophilic group appears to have a relatively slight effect, no significant difference being noted between peptides derived from stearylamine and cetylamine. Branching in the alkyl group appears to decrease water solubility. Although there is no definite correlation between water solubility and emulsifying power, in this series, at least, the most water soluble are the most satisfactory emulsifying agents. None of the present peptides when tested alone produces emulsions stable for an appreciable time, but in conjunction with other emulsifying agents, excellent, stable emulsions of Nujol and water are obtained. In our own experiments we used cholesterol or the monostearyl ether of ethylene glycol (see Experimental section). Of the peptides examined N-[L-alanyl]-octadecylamine is the most satisfactory. Preliminary tests of

(10) Based on the procedure of E. Chargaff and M. Bovarnick, *J. Biol. Chem.*, **118**, 431 (1937), for the preparation of the N-carbobenzoxy derivative VIII, R = $CH_2C_6H_5$.

this substance with two commercial coemulsifiers (polyoxyethylene type and tartaric acid ester type) indicate that it has promising emulsifying properties.[11]

We also investigated the properties of amino acids of the type $RCH(NH_2)COOH$, such as α-aminomyristic acid, α-aminopalmitic acid and α-aminostearic acid, with the expectation that the dipolar-ion structure would confer water solubility, as in the natural amino acids. These α-amino derivatives of fatty acids have practically no solubility in water or in the usual organic solvents, as already reported.[12]

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Experimental[13]

Octadecanyl Phosphorylcholine (Y. H.).—Octadecanol (10.8 g.) dissolved in chloroform (180 ml.) was added slowly to a vigorously stirred and cooled (10°) solution of monophenylphosphoryl dichloride (6 ml.) in chloroform (16 ml.) and pyridine (3.4 ml.). The temperature of the water-bath was then raised to 35°; after ten minutes 50 ml. of pyridine was added and then 5.6 g. of choline chloride (powdered and well dried). The mixture was then stirred at room temperature for 48 hr. The solvent was removed under reduced pressure and the residue extracted three times with ether (50 ml.). The ether-insoluble material was dissolved in 50 ml. of water, the solution was saturated with sodium chloride and the phosphorylation product extracted by chloroform. After removal of the solvent the product was crystallized several times from acetone, but was not obtained analytically pure; m.p. 82–86°, yield 7 g.

Octadecanyl phosphorylcholine chloride was obtained by catalytic hydrogenation of the above product (3.0 g., PtO_2, ethanol), m.p. 71–72° (acetone), yield 1.8 g.

Anal. Calcd. for $C_{23}H_{51}O_4PNCl$: C, 58.52; H, 10.89. Found: C, 58.60; H, 10.23.

The chloride was converted into the **hydroxide** by treatment of an alcoholic solution with Amberlite IRA-400. The solvent was then partially removed, and the phosphorylcholine ester precipitated by addition of acetone. The crude product could be purified by chromatography, using chloroform–ethanol (4:1) as eluent. After two crystallizations from chloroform, it melted over a range (220–230°).

Anal. Calcd. for $C_{23}H_{50}O_5PN$: C, 60.89; H, 11.55; P, 6.82; N, 3.08. Found: C, 60.94; H, 11.62; P, 6.84; N, 2.95.

This substance is sparingly soluble in water and in Nujol at room temperature; it shows no emulsifying properties.

Dihydrophytyl phosphorylcholine and cholestanyl phosphorylcholine were prepared by essentially the same procedure, but we were unable to obtain analytically pure samples in either series. The crude dihydrophytyl derivative (semi-solid) showed some emulsifying action, but was inferior to soya bean phosphatide.

N-Alkylarabonamides, $CH_2OHCH(OH)—CH(OH)CHCONHR$ (with OH)

(E. T.).—An aqueous solution of L-arabinose (75 g.) was oxidized with bromine (120 g., 50% excess) at room temperature for 12 hr. according to the general procedure of Kiliani and Kleeman.[14] The excess bromine was removed under reduced pressure at 40–50° and lead oxide (120 g.) was added to the reaction. After several hours the white precipitate (lead bromide) was removed and sulfuric acid was added dropwise to the solution until a test of the filtered solution shows no further precipitate. The filtered solution was concentrated under reduced pressure at 50° and methanol (75 ml.) was then added. After a few hours standing at 5° crystals separated. Crystallized from methanol the ester melts at 148–150°, $\alpha^{30}D$ −6.5° (lit.[15] m.p. 143°, αD −6.7, −5.7), over-all yield 77%. The condensation of the ester with *n*-laurylamine is a typical procedure. The ester (2 g.) in methanol (100 ml.) was treated with the amine (2.2 g., 10% excess). The mixture was warmed sufficiently to dissolve the amine and then allowed to stand at room temperature. The crude product (87.5% yield) separated after a few hours and was purified by crystallization from ethanol or dioxane.

N-Decylarabonamide, R = $C_{10}H_{21}$, m.p. 150–151° (alcohol). *Anal.* Calcd. for $C_{15}H_{31}NO_5$: C, 58.98; H, 10.23; N, 4.58. Found: C, 58.83; H, 10.23; N, 4.59.

N-Laurylarabonamide, R = $C_{12}H_{25}$, m.p. 150–151°. *Anal.* Calcd. for $C_{17}H_{35}NO_5$: C, 61.22; H, 10.58; N, 4.20. Found: C, 60.33; H, 10.53; N, 4.28.

N-Myristylarabonamide, R = $C_{14}H_{29}$, m.p. 150–151° (alcohol). *Anal.* Calcd. for $C_{19}H_{39}NO_5$: C, 63.12; H, 10.87; N, 3.87. Found: C, 63.57; H, 11.08; N, 3.95.

N-Cetylarabonamide, R = $C_{16}H_{33}$, m.p. 150–151° (dioxane). *Anal.* Calcd. for $C_{21}H_{43}NO_5$: C, 64.74; H, 11.13; N, 3.60. Found: C, 64.83; H, 11.04; N, 3.78.

N-Stearylarabonamide, R = $C_{18}H_{37}$, m.p. 149–150° (dioxane). *Anal.* Calcd. for $C_{23}H_{47}NO_5$: C, 66.15; H, 11.34; N, 3.35. Found: C, 66.34; H, 11.38; N, 3.80.

The first two members of the series have slight water solubility; the others are practically insoluble in both water and Nujol.

N-Alkylgluconamides, $CH_2OHCH(OH)CH(OH)CH(OH)CHCONHR$ (with OH)

(M. T. and H. H.).—Condensation of the lactone of gluconic acid with stearyl and similar amines at 140°[16] led to waxy products. The reaction is improved by carrying out the condensation in refluxing ethanol. In a typical experiment the lactone (1 g.) and the theoretical amount of the amine were heated in refluxing ethanol for one hour. The amides separated on cooling and were purified by crystallization from the same solvent.

N-Laurylgluconamide, R = $C_{12}H_{25}$, m.p. 153.2–155.6°. *Anal.* Calcd. for $C_{18}H_{37}O_6N$: C, 59.48; H, 10.26; N, 3.85. Found: C, 59.58; H, 10.37; N, 4.03.

N-Cetylglyconamide, R = $C_{16}H_{33}$, m.p. 150.4–154.6°. *Anal.* Calcd. for $C_{22}H_{45}O_6N$: C, 62.97; H, 10.81; N, 3.34. Found: C, 63.29; H, 10.83; N, 3.51.

N-Stearylgluconamide, R = $C_{18}H_{37}$, m.p. 149.4–154.8°. *Anal.* Calcd. for $C_{24}H_{49}O_6N$: C, 64.4; H, 11.1; N, 3.1. Found: C, 64.37; H, 11.02; N, 2.95.

N-Stearylglucoheptonamide was prepared similarly from 2.08 g. of glucoheptonolactone (m.p. 148–152°) and 2.69 g. of stearylamine. The product separated on cooling (55% yield). An analytical sample was obtained by recrystallization with ethanol from a Soxhlet thimble, m.p. 149–152° (cloudy), dec. about 180°.

Anal. Calcd. for $C_{25}H_{51}O_7N$: C, 62.86; H, 10.76; N, 2.93. Found: C, 62.65; H, 10.74; N, 2.88.

The C_{14}-, C_{16}- and C_{18}-alkyl derivatives of gluconamide have some solubility in water (about 6 g./l. in boiling water); when used with cholesterol or the monostearyl ether of ethylene glycol (preparation, see below) emulsions with an average particle size of 5–10 μ can be obtained (using a Waring blender). These emulsions are stable for only a few hours.

N-Alkyl-1,2-isopropylideneglucuronamides (II) (E. T.).—1,2-Isopropylideneglucuronolactone (I) was prepared in 81% yield by a slight modification of the literature procedure[8]; the volume of acetone was reduced to 500 ml. for 20 g. of

(11) Conducted by the Southern Utilization Research Branch of the U. S. Dept. of Agriculture.

(12) C. Hell, *et al.*, *Ber.*, **22**, 1745 (1889); **24**, 936, 2388 (1891).

(13) Analyses by S. M. Nagy and associates.

(14) H. Kiliani and S. Kleeman, *Ber.*, **17**, 1296 (1884).

(15) K. H. Böddener and B. Tollens, *ibid.*, **43**, 1649 (1910).

(16) Based on procedures of Th. W. T. van Marle [*Rec. trav. chim.*, **39**, 549 (1920)] and of W. E. van Wijk [*ibid.*, **40**, 221 (1921)].

glucuronolactone, and sodium carbonate was used instead of barium carbonate for neutralization of the acid catalyst.

1,2-Isopropylideneglucuronamide (II, R = H) was prepared from I (6.6 g.), dissolved in 50 ml. of dioxane, by the addition of 15 ml. of ice-cold, concd. aqueous ammonia. After the reaction had stood for 4–5 hr. in the cold room the solution was evaporated under reduced pressure at a temperature of 45° or less. The product is obtained in nearly quantitative yield as needles from absolute methanol, m.p. 163–164°, $\alpha^{15}D$ −13.5° (*c* 1, water).

1,2-Isopropylidene-N-stearylglucuronamide (II, R = $C_{18}H_{37}$).—The lactone I (5.8 g.) was dissolved in 50 ml. of pure, dry tetrahydrofuran and then treated with stearylamine (6.8 g., 10% excess), added in small portions and with agitation. The solution was kept overnight in the cold room and then allowed to stand at room temperature for a few hours. The solvent was removed under reduced pressure (temperature below 40°) to the point of incipient crystallization, and petroleum ether was then added. The solid product was washed with petroleum ether; m.p. 92–93°, 8.0 g. Further concentration of the mother liquors yielded additional material, m.p. 86–90°, 2.3 g.

Anal. Calcd. for $C_{24}H_{51}O_6N$: C, 66.78; H, 10.58; N, 2.88. Found: C, 66.93; H, 10.40; N, 3.21.

The same procedure was used for the preparation of the following N-alkylamides, yields 70–90%.

1,2-Isopropylidene-N-decylglucuronamide (II, R = $C_{10}H_{21}$), m.p. 70–75° (petroleum ether), αD −14° (*c* 1.162, methanol). *Anal.* Calcd. for $C_{19}H_{35}O_6N$: N, 3.75. Found: N, 3.96.

1,2-Isopropylidene-N-laurylglucuronamide (II, R = $C_{12}H_{25}$), m.p. 87–88° (methanol), αD −13° (*c* 1.046, methanol). *Anal.* Calcd. for $C_{21}H_{39}O_6N$: C, 62.81; H, 9.79; N, 3.49. Found: C, 63.01; H, 9.81; N, 3.72.

1,2-Ispropylidene-N-myristylglucuronamide (II, R = $C_{14}H_{29}$), m.p. 88–90° (methanol), αD −12.5° (*c* 1.09, methanol). *Anal.* Calcd. for $C_{23}H_{43}O_6N$: C, 64.30; H, 10.09; N, 3.2. Found: C, 64.59; H, 10.19; N, 3.59.

1,2-Isopropylidene-N-cetylglucuronamide (II, R = $C_{16}H_{31}$), m.p. 90–92° (ethanol), αD −13.5° (*c* 1.064, methanol). *Anal.* Calcd. for $C_{25}H_{47}O_6N$: C, 65.61; H, 10.35; N, 3.06. Found: C, 65.75; H, 10.24; N, 3.56.

1,2-Isopropylidene-N-(ω)-cyclohexyldecylglucuronamide (II, R = C_6H_{11}-$C_{10}H_{20}$).—ω-Cyclohexyldecanoic acid (10 g.) was refluxed for two hours with thionyl chloride (15 ml.); excess reagent was removed under reduced pressure and the cooled residue was poured slowly into an ice-cold solution of concd. aqueous ammonia (100 ml.). The solid **ω-cyclohexyldecanamide** was collected after one hour and crystallized from methanol–water; m.p. 89–93°, 9 g. (90% yield).

Anal. Calcd. for $C_{16}H_{31}ON$: N, 5.53. Found: N, 5.67.

The amide (7.6 g.) was reduced in the usual way with lithium aluminum hydride in refluxing ether and the product, **ω-cyclohexyldecylamine**, isolated as the hydrochloride, m.p. 151–153° (methanol), 6.3 g. (76% yield). The free amine melts at about 50°.

Anal. Calcd. for $C_{16}H_{34}NCl$: N, 5.07. Found: N, 5.39.

An ethereal solution of the free amine (liberated with aqueous bicarbonate from the hydrochloride) was treated with the lactone I to give the desired amide II, m.p. 88–90°.

Anal. Calcd. for $C_{25}H_{45}O_6N$: C, 65.90; H, 9.96; N, 3.07. Found: C, 65.51; H, 9.99; N, 3.08.

Glucuronamide (III, R = H, E. T.).—The 1,2-isopropylidine derivative II (R = H, 2.3 g.) was dissolved in 20 ml. of water and 0.5 ml. of concd. hydrochloric acid and then heated on the steam-bath at 80° for 1–3 min. The water was removed under reduced pressure and the amide purified by crystallization from absolute methanol (10–15 ml., 5°), m.p. 159–160° dec., 1.8 g., 85% yield. Recrystallized material decomposes sharply at 168–169°, positive Fehling test, $\alpha^{22}D$ +70° → +31.9° (44 hr., *c* 1.77 water). This material is a hydrate[9]; anhydrous material, m.p. 173–174°, is obtained by drying in vacuum at 100° for several hours.

Anal. Calcd. for $C_6H_{11}O_6N$: C, 37.31; H, 5.74; N, 7.25. Found: C, 37.61; H, 5.93; N, 6.96.

The same material is obtained from the γ-lactone of β-methylglucuronoside (IV) as follows. The lactone (4.2 g.), dissolved in cold dioxane (20 ml.), was treated overnight with an ice-cold aqueous solution of ammonia (10 ml., d. 0.9). The solvent was removed under reduced pressure at 45° and the amide V was hydrolyzed with hydrochloric acid under the conditions used for the isopropylidene derivatives. The crude glucuronamide (2.1 g., 73% yield) was purified as described above.

N-Alkylglucuronamides (III, E. T.).—All the N-alkylamides were obtained by hydrolysis of the 1,2-isopropylidine derivatives (II) under the following conditions. A suspension of 5 g. of II in 100–350 ml. of water[17] and 7 ml. of concd. hydrochloric acid was stirred and heated on the steam-bath until a homogeneous colloidal solution was obtained (30–45 min.). The free alkyl amides separated on cooling and were purified by crystallization from the solvents indicated after the melting points. They are white, crystalline solids melting about 150°, positive Fehling test; they exhibit mutarotation.

N-Decylglucuronamide (III, R = $C_{10}H_{21}$), m.p. 145–148° dec., methanol–water, αD +24° (*c* 1.11, methanol). *Anal.* Calcd. for $C_{16}H_{31}O_6N$: C, 57.63; H, 9.37; N, 4.20. Found: C, 57.53; H, 9.69; N, 4.37.

N-Laurylglucuronamide (III, R = $C_{12}H_{25}$), m.p. 160–161° (dioxane–water), αD −4° → +22° (24 hr., *c* 1.18, methanol). *Anal.* Calcd. for $C_{18}H_{35}O_6N$: C, 59.81; H, 9.76; N, 3.88. Found: C, 60.01; H, 9.84; N, 4.06.

N-Myristylglucuronamide (III, R = $C_{14}H_{29}$), m.p. 156–157° (dioxane–water), αD +11° → +24° (24 hr., *c* 1.05, methanol). *Anal.* Calcd. for $C_{20}H_{39}O_6N$: C, 61.67; H, 10.09; N, 3.60. Found: C, 62.07; H, 10.20; N, 3.53.

N-Cetylglucuronamide (III, R = $C_{16}H_{33}$), m.p. 155–157° (dioxane–water), αD +24.7° → +26° (24 hr., *c* 1.03, methanol). *Anal.* Calcd. for $C_{22}H_{43}O_6N$: C, 63.28; H, 10.38; N, 3.35. Found: C, 63.76; H, 10.17; N, 3.64.

N-Stearylglucuronamide (III, R = $C_{18}H_{37}$), m.p. 153–154° (dioxane–water), αD +23° (10 min., *c* 1.046 methanol). *Anal.* Calcd. for $C_{24}H_{47}O_6N$: C, 64.68; H, 10.63; N, 3.14. Found: C, 64.94; H, 10.46; N, 3.14.

N-ω-Cyclohexyldecylglucuronamide (III, R = C_6H_{11}-$(CH_2)_{10}$), m.p. 128–130° (methanol), αD +21° → +25° (24 hr., *c* 1.15, methanol). *Anal.* Calcd. for $C_{22}H_{41}O_6N$: C, 63.58; H, 9.95; N, 3.37. Found: C, 63.22; H, 10.01; N, 3.51.

N-ω-Cyclohexylbutylglucuronamide (III, R = C_6H_{11}-$(CH_2)_4$) was prepared by the same procedure used for the amide described above but using ω-cyclohexylbutyramide (m.p. 103–106°. *Anal.* C, 70.39; H, 10.40; N, 8.60. Calcd. for $C_{16}H_{17}ON$: C, 70.96; H, 11.32; N, 8.28) and ω-cyclohexylbutylamine hydrochloride (m.p. 165–167°, *Anal.* C, 62.39; H, 10.81; N, 7.37. Calcd. for $C_{10}H_{22}NCl$: C, 62.63; H, 11.57; N, 7.30). The condensation of the free amine with the glucuronolactone derivative was carried out in the usual manner, 80% yield, m.p. 160–163° methanol–water), αD +35.8° → +23.5° (24 hr., *c* 1.54, methanol).

Anal. Calcd. for $C_{16}H_{29}O_6N$: C, 57.98; H, 8.82; N, 4.23. Found: C, 58.30; H, 9.03; N, 4.22.

The N-cetyl-, N-stearyl- and ω-cyclohexyldecylglucuronamides give fairly stable oil-in-water emulsions when used with a coemulsifier.

N-Alkylamides of β-Methylglucuronoside (V, E. T.).—As mentioned in the theoretical section the substances were obtained in two different forms. We did not investigate fully the experimental conditions favoring one or the other form, but, in general, condensations conducted at temperatures of the cold room lead to the low-melting form, while those at 40–50° furnished the high-melting form. Typical experimental procedures are described for the **N-stearylamide** (V, R = $C_{18}H_{37}$).

Form A: A cold solution of stearylamine (2.5 g., theory 2.7 g.) in tetrahydrofuran (15 ml.) was added to a cold solution of β-methylglucuronoside-γ-lactone[3] (2 g.) in the same solvent, and the mixture was allowed to stand overnight in the cold room and then at room temperature for 1–2 hr. The solvent was removed under reduced pressure, and the residue crystallized from ether (30 ml.); 77% yield, m.p. 75–78°, $\alpha^{21}D$ −60.4° (*c* 1.03, methanol).

Anal. Calcd. for $C_{25}H_{49}ON$: C, 65.32; H, 10.75; N, 3.05. Found: C, 65.50; H, 10.75; N, 3.28.

Form B: The above condensation was carried out in the same solvent but at an initial temperature of 40–50° for 0.5

(17) The amount of water necessary depends upon the size of the alkyl group: 100 ml. for R = *n*-$C_{10}H_{21}$—350 cc. for R = *n*-$C_{18}H_{37}$, with intermediate amounts for intermediate R groups.

hour and then at 25° for 2–3 hr. The solvent was removed as before and the product washed with petroleum ether and then crystallized from methanol–benzene; 87% yield, $\alpha^{25}D$ −60.7° (*c* 1.0, methanol), m.p. 93–95°, intermediate mixed melting points with form A.

Anal. Found: C, 65.39; H, 10.50; N, 3.02.

N-Lauryl-β-methylglucuronamidoside (V, R = $C_{12}H_{25}$); form A, m.p. 68–70°, αD −58.4° (*c* 1.05 methanol); form B: m.p. 88–90°, αD −58.7° (*c* 1.43, methanol). *Anal.* Calcd. for $C_{19}H_{37}O_6N$: C, 60.77; H, 9.93; N, 3.73. Found, form A: C, 61.11; H, 9.80; N, 3.68; form B: C, 61.03; H, 9.40; N, 3.87.

N-Myrystyl-β-methylglucuronamidoside (V, R = $C_{14}H_{29}$); form A: m.p. 70–73°, αD −60.8° (*c* 1.11, methanol); form B: m.p. 88–90°, αD −61° (*c* 1.04, methanol). *Anal.* Calcd. for $C_{21}H_{41}O_6N$: C, 62.49; H, 10.24; N, 3.47. Found, form A: C, 62.41; H, 10.06; N, 3.39; form B: C, 62.87: H, 10.34; N, 3.47.

N-Cetyl-β-methylglucuronamidoside (V, R = $C_{16}H_{33}$); form A: m.p. 75–78°, αD −60.6° (*c* 1.3, methanol); form B: m.p. 92–93°, αD −60.5° (*c* 1.3, methanol). *Anal.* Calcd. for $C_{23}H_{45}O_6N$: C, 64.00; H, 10.51; N, 3.24. Found, form A: C, 64.06; H, 10.6; N, 3.31; form B: C, 64.11; H, 10.20; N, 3.37.

The glucuronosides V were hydrolyzed to the glucuronamides III with dilute hydrochloric acid (1 ml. of concd. acid in 100 ml. of water) at steam-bath temperature, 10 min. The products crystallized on cooling in nearly quantitative yields.

N-Alkylglucosaccharonamides (VI, E. T.).—These were prepared by bromine oxidation of the corresponding N-alkylglucuronamide (III) under the following conditions in which the only variation was the amount of solvent used. The amide (5 g.) was dissolved in 250–500 ml. of hot water[18]; the solution was cooled to 50–60° and treated with 4 ml. of bromine with cooling required to maintain the temperature at 40–50°. After 15–30 minutes the bromine color was discharged and the solution was then kept in the cold room overnight. Excess bromine was eliminated by addition of a saturated solution of sodium sulfite. The product was air-dried and then crystallized from tetrahydrofuran in yields about 80%.

N-Laurylglucosaccharonamide (VI, R = $C_{12}H_{25}$), m.p. 134–137°, αD −21.5° (*c* 1.13, tetrahydrofuran). *Anal.* Calcd. for $C_{18}H_{35}O_7N$: C, 57.27; H, 9.35; N, 3.71. Found: C, 56.86; H, 9.30; N, 3.63.

N-Myristylglucosaccharonamide (VI, R = $C_{14}H_{29}$), m.p. 125–127°, αD −22° (*c* 1.06, tetrahydrofuran). *Anal.* Calcd. for $C_{20}H_{39}O_7N$: C, 59.23; H, 9.69; N, 3.45. Found: C, 59.46; H, 9.61; N, 3.50.

N-Cetylglucosaccharonamide (VI, R = $C_{16}H_{33}$), m.p. 135–138° (previous sintering), αD −21° (*c* 1.14, tetrahydrofuran). *Anal.* Calcd. for $C_{23}H_{43}O_7N$: C, 60.94; H, 10.00; N, 3.23. Found: C, 60.84; H, 10.02; N, 3.20.

N-Stearylglucosaccharonamide (VI, R = $C_{18}H_{37}$), m.p. 137–139°, αD −22° (*c* 1.12, tetrahydrofuran). *Anal.* Calcd. for $C_{24}H_{27}O_7N$: C, 62.44; H, 10.26; N, 3.03. Found: C, 62.75; H, 10.41; N, 3.12.

These saccharonamides give less stable emulsions than the corresponding glucuronamides; they have slightly greater water solubility.

N-Acylglucosamines, VIII (E. T.).—The preparation used for the **N-lauroyl** derivative (VIII, R = $C_{11}H_{23}$) is typical for the other members of the series. A solution of lauroyl chloride (2.2 g.) in tetrahydrofuran (20 ml.) was added dropwise with stirring to a solution of glucosamine hydrochloride (2.15 g.) and sodium bicarbonate (2 g.) in 20 ml. of water. Use of a vibro mixer proved advantageous. Agitation was continued for 30 min. after the addition was completed and the product was then precipitated by addition of 100 ml. of water. It was purified by washing with water and crystallization from dioxane–ethanol; 3.2 g. (88% yield), m.p. 190–193° dec.

Anal. Calcd. for $C_{18}H_{35}O_6N$: C, 59.81; H, 9.76; N, 3.88. Found: C, 59.50; H, 9.90; N, 3.82.

N-Myrlstoylglucosamine (VIII, R = $C_{13}H_{27}$), m.p. 193–195° dec., dioxane–ethanol. *Anal.* Calcd. for $C_{20}H_{39}O_6N$: C, 61.67; H, 10.09; N, 3.60. Found: C, 61.13; H, 10.05; N, 3.51.

(18) The first figure corresponds to the amount used for the laurylamide and the second for the stearylamide, intermediate amounts being used for amides substituted by intermediate R groups.

N-Palmitoylglucosamine (VIII, R = $C_{15}H_{31}$), m.p. 190–193° dec., dioxane–ethanol. *Anal.* Calcd. for $C_{22}H_{43}O_6N$: C, 63.28; H, 10.48; N, 3.35. Found: C, 62.75; H, 10.41; N, 2.83.

N-Stearoylglucosamine (VIII, R = $C_{17}H_{35}$), m.p. 190–191° dec., dioxane–ethanol. *Anal.* Calcd. for $C_{24}H_{47}O_6N$: C, 64.68; H, 10.63; N, 3.14. Found: C, 64.65; H, 10.62; N, 3.11.

Stearoylamino Acids (E. T. and S. B.).—These were prepared by the now standard mixed anhydride method, involving condensation of the amino acid with the mixed anhydride of stearic acid and ethyl chlorocarbonate. The experimental procedures used were based on those described by Vaughan and Eichler.[19]

Stearoyl-β-alanine, $C_{17}H_{35}CONHCH_2CH_2COOH$.—To a well-stirred solution of stearic acid (11.4 g.) and triethylamine (6 ml.) in dry tetrahydrofuran cooled to −5° was added ethyl chlorocarbonate (4 ml.) and then, after five minutes and without further cooling, an ice-cold solution of the sodium salt of β-alanine, prepared from β-alanine (3.6 g.) and sodium hydroxide (1.6 g.) dissolved in 30 ml. of water. The reaction was stirred for 30 min. and then acidified to *p*H 3–4. The precipitated peptide was collected, washed well with warm water and dried. Purification involved extraction of unchanged stearic acid with petroleum ether followed by crystallization from dioxane–water (4:1) or tetrahydrofuran; 11.2 g. (71%), m.p. 122–124°, insoluble in water at 25°, somewhat soluble at 100°.

Anal. Calcd. for $C_{21}H_{41}O_3N$: C, 70.94; H, 11.62; N, 3.94. Found: C, 70.96; H, 11.57; N, 3.96.

Oleoyl-β-alanine was prepared in the same manner, m.p. 75–76° (dioxane–water). *Anal.* Calcd. for $C_{21}H_{39}O_3N$: C, 71.34; H, 11.12; N, 3.96. Found: C, 71.07; H, 10.80; N, 4.04.

9,10-Dihydroxystearoyl-β-alanine.—A stirred mixture of oleoyl-β-alanine (1 g.), silver acetate (1.2 g.) and glacial acetic acid (13 ml.) containing water (0.1 ml.) was treated with iodine (0.72 g.), added over a period of 40 min. The mixture was then heated for 3 hr. on the steam-bath. The cooled, filtered solution was evaporated under suction; a methanolic solution of the residue was refluxed with aqueous potassium hydroxide for 25 min. After removal of some solid, the filtrate was acidified and the precipitated product purified by crystallization from ethanol; m.p. 148–150°, yield 0.6 g.

Anal. Calcd. for $C_{21}H_{41}O_5N$: C, 65.08; H, 10.66; N, 3.63. Found: C, 65.24; H, 10.72; N, 3.72.

Stearoyl-β-alanyl-β-alanine.—An additional amino acid residue was added by the same procedure using stoichiometric amounts of reagents; 71% yield, m.p. 153–156° (dioxane–water, 3:2), similar to N-stearoyl-β-alanine in water solubility. *Anal.* Calcd. for $C_{24}H_{46}O_4N_2$: C, 67.56; H, 10.87; N, 6.57. Found: C, 67.45; H, 10.82; N, 6.79.

Stearoyl-β-alanylglycine was prepared by the standard method from N-stearoyl-β-alanine and glycine in 75% yield, m.p. 172–174° (dioxane–water). *Anal.* Calcd. for $C_{23}H_{44}O_4N_2$: C, 66.95; H, 10.75; N, 6.79. Found: C, 66.97; H, 10.80; N, 6.60.

Stearoyl-β-alanyltaurine was obtained in 78% yield; recrystallized from water apparently with solvent of crystallization which is not removed on drying at 150°, melts with decomposition about 200°. *Anal.* Calcd. for $C_{23}H_{46}O_5N_2S\cdot 3H_2O$: C, 53.46; H, 10.12; N, 5.42. Found: C, 53.66; H, 9.50; N, 5.70.

DL-Stearoyl-α-alanine.—Stearic acid (3 g.) and triethylamine (1.07 g.) in chloroform–ethyl acetate solution were treated in the usual way with isobutyl chlorocarbonate (1.44 g.), the ethyl ester of alanine hydrochloride (1.62 g.) and triethylamine (1.07 g.). **Stearoyl-α-alanine ethyl ester** (2.87 g.) was purified by crystallization from ligroin, m.p. 62–65°.

Anal. Calcd. for $C_{23}H_{45}O_3N$: C, 72.01; H, 11.82; N, 3.65. Found: C, 72.27; H, 11.92; N, 3.64.

The ester (1 g.) in dioxane (10 ml.) was hydrolyzed with concd. hydrochloric acid (3 cc. in 1.5 cc. of water) at steam-bath temperature for 1 hr. Stearoyl-α-alanine (0.63 g.) melts at 115–117° (ligroin–dioxane).

Anal. Calcd. for $C_{21}H_{41}O_3N$: C, 70.94; H, 11.62; N, 3.94. Found: C, 70.88; H, 11.58; N, 3.88.

(19) J. R. Vaughan, Jr., and J. A. Eichler, THIS JOURNAL, **76**, 2474 (1954).

Stearoyl-α-alanyl-α-alanine ethyl ester was prepared by the mixed anhydride method from stearoyl-α-alanine and the ethyl ester of alanine; m.p. 82–83°.

Anal. Calcd. for $C_{26}H_{50}O_4N_2$: C, 68.68; H, 11.08; N, 6.16. Found: C, 68.38; H, 11.15; N, 6.04.

Acid hydrolysis gave the free **acid**, m.p. 132–133°. Repeated crystallizations (petroleum ether–dioxane) failed to give an analytically pure sample [*Anal.* Found: C, 68.36; H, 11.02; N, 6.12. Calcd. for $C_{24}H_{48}O_4N_2$: C, 67.56; H, 10.87; N, 6.57].

Stearoylglycine has been prepared previously in unspecified yield by the acid chloride method.[20] It is obtained in 75–80% yield by the mixed anhydride method; m.p. 125–127° (ethyl acetate–tetrahydrofuran).

Stearoylglycyl-β-alanine, 70–75% yield, m.p. 169–170° (dioxane). *Anal.* Calcd. for $C_{23}H_{44}O_4N_2$: C, 66.95; H, 10.75; N, 6.79. Found: C, 66.82; H, 10.68; N, 6.67.

Stearoylglycylglycine, 75–80% yield; m.p. 170–172° (dioxane), lit. 178°.[20]

Stearoylglycyltaurine was obtained crude in 80–90% yield. It is practically insoluble in various organic solvents. Crystallized from water, it apparently contains water of crystallization, which is not lost on drying at 150°. *Anal.* Calcd. for $C_{22}H_{44}O_5N_2S\cdot H_2O$: C, 56.63; H, 9.94; N, 6.00. Found: C, 57.05; H, 9.70; N, 5.82 (residue).

Stearoyltaurine ($C_{17}H_{35}CONHCH_2CH_2SO_3H$) was prepared in the usual way, crystallized from water, 73% yield, decomposes about 240°. *Anal.* Calcd. for $C_{20}H_{41}NS\cdot 1^1/_2H_2O$: C, 57.3; H, 10.6; N, 3.3. Found: C, 57.34; H, 9.6; N, 3.35.

Stearoyl-DL-aspartic acid, $C_{17}H_{35}CONH(COOH)CH_2COOH$, was obtained in yields of only about 50% by the standard mixed anhydride procedure using aspartic acid. It is more advantageously prepared by hydrolysis of stearoyl-DL-asparagine, $C_{17}H_{35}CONHCH(COOH)CH_2CONH_2$. This amide derivative is obtained in 70% yield by condensation of stearic acid with asparagine by the mixed anhydride procedure; m.p. 145–148° (dioxane). *Anal.* Calcd. for $C_{23}H_{44}O_4N_2$: C, 66.29; H, 10.62; N, 7.03. Found: C, 66.01; H, 10.67; N, 6.70.

The free acid was obtained by treating the amide (0.4 g.) dissolved in dioxane (10 ml.) with sodium nitrite (0.08 g.) dissolved in water (30 ml.); concd. hydrochloric acid (0.4 ml.) was added and the mixture warmed on the steam-bath for 4–6 hr. until the solid amide had disappeared. When allowed to cool to room temperature the solution slowly deposits crystals of the free acid, 0.37 g., m.p. 111–113°. When prepared directly the acid was purified by crystallization from dioxane–water and from ethyl acetate.

Anal. Calcd. for $C_{23}H_{43}O_5N$: C, 66.13; H, 10.34; N, 3.51. Found: C, 65.98; H, 10.59; N, 3.51.

It is converted into the **anhydride** when heated for 15 min. at 70–80° in acetic anhydride and crystallizes from the cooled solution; m.p. 124–125° (ligroin containing some tetrahydrofuran), yield quantitative. *Anal.* Calcd. for $C_{22}H_{39}O_4N$: C, 69.25; H, 10.30; N, 3.67. Found: C, 68.67; H, 10.36; N, 3.96.

Stearoyl-L-glutamic acid was prepared by the mixed anhydride procedure from L-glutamic acid in 55% yield, m.p. 127–128° (tetrahydrofuran), $\alpha^{22}D$ +8.5° (*c* 1.62, dioxane). *Anal.* Calcd. for $C_{23}H_{43}O_5N$: C, 66.79; H, 10.48; N, 3.39. Found: C, 66.77; H, 10.56; N, 3.35.

The anhydride was prepared as in the case of the aspartic acid derivative, m.p. 107–109° (ligroin–tetrahydrofuran). *Anal.* Calcd. for $C_{23}H_{41}O_4N$: C, 69.83; H, 10.45; N, 3.54. Found: C, 69.14; H, 10.23; N, 3.51.

Stearoylglycylasparagine, 70–75% yield, m.p. 180–185° (dioxane–water). *Anal.* Calcd. for $C_{24}H_{45}O_5N_3\cdot{}^1/_2H_2O$: C, 62.04; H, 9.98; N, 9.04. Found: C, 61.83; H, 10.05; N, 8.91.

Stearoylglycyl-DL-aspartic acid was obtained in 80–90% yield from the amide by acid hydrolysis in the presence of nitrous acid as described above; m.p. 165–170° (acetone–water). It was also prepared directly from stearoylglycine and aspartic acid (40–60% yield).

Anal. Calcd. for $C_{24}H_{44}O_6N_2$: C, 63.13; H, 9.71; N, 6.14. Found: C, 63.18; H, 9.90; N, 6.12.

Anhydride, m.p. 175–180°. *Anal.* Calcd. for $C_{24}H_{42}O_5N_2$: N, 6.39. Found: N, 6.59.

(20) A. Koebner, *J. Chem. Soc.*, 564 (1941).

α-Aminostearoyl-DL-α-alanine (S. B.).—The phthalimido derivative of α-aminostearic acid[21] was prepared by heating the amino acid with phthalic anhydride at 145–160° for 30 minutes, m.p. 81° (ligroin). *Anal.* Calcd. for $C_{36}H_{39}O_4N$: C, 72.69; H, 9.15; N, 3.25. Found: C, 72.72; H, 9.34; N, 3.22

This derivative (2 g.) was refluxed with thionyl chloride (10 ml.) for 3 hr. Excess reagent was removed under suction and the residual oil washed with dry toluene and then dried (1 mm. pressure). To the acid chloride dissolved in dry chloroform (20 ml.) was added the ethyl ester of α-alanine hydrochloride (0.71 g.) in dry chloroform (10 ml.), and the mixture was cooled to −20°. Triethylamine (1.1 g.) in dry chloroform was then added to the stirred cooled reaction mixture over a period of 40 min. The reaction was then allowed to come to room temperature and the solvent was removed under reduced pressure. The residue was taken up in ligroin and washed well with water to remove the catalyst. On evaporation of the ligroin and addition of petroleum, **α-phthalimidostearoyl-α-alanine ethyl ester** was obtained in crystalline form, m.p. 63–64°, yield 0.9 g.

Anal. Calcd. for $C_{31}H_{48}O_5N_2$: C, 70.45; H, 9.09; N, 5.3. Found: C, 70.10; H, 8.93; N, 5.21.

The free acid was obtained by acid hydrolysis in the usual way (dioxane, water, HCl); m.p. 116° (ligroin).

Anal. Calcd. for $C_{29}H_{44}N_2O_5$: C, 69.60; H, 8.80; N, 5.60. Found: C, 69.10; H, 8.75; N, 5.53.

The phthalimido group was removed by refluxing the above acid (0.45 g.) in 95% ethanol (7 ml.) with hydrazine (1.5 cc.) and a few drops of water for 45 min. On cooling and addition of water, α-aminostearoyl-α-alanine separated as crystals, m.p. 218–220°, 0.28 g. An analytical sample was obtained by washing the crystals with boiling ethanol.

Anal. Calcd. for $C_{21}H_{42}N_2O_3$: C, 68.06; H, 11.42; N, 7.56. Found: C, 68.10; H, 11.54; N, 7.65.

Dipeptides of the Type $RNHCOCH(NH_2)R'$ (E. T.).—The general procedure involved condensation of stearylamine (or palmitylamine) with the N-carbobenzoxy derivative of the amino acid using the mixed anhydride method (ethyl chlorocarbonate, triethylamine). Yields in the condensation are in the order of 70%. The last step involved removal of the protective group by hydrogenation (10% Pd-on-carbon, quantitative yield). The following preparation of **DL-alanylstearylamine**, R = $C_{18}H_{37}$, R′ = CH_3, is a typical procedure. N-Carbobenzoxy-DL-alanine[22] (4.46 g., m.p. 120–122°) was dissolved in dry tetrahydrofuran (50 ml.) containing triethylamine (3 ml.). The solution was cooled to −5° and stirred with a vibromixer during the addition of stearylamine (5.4 g.) dissolved in tetrahydrofuran (50 ml.). The reaction was stirred for a further 30 min. without cooling and then acidified. The solvent was partially removed under reduced pressure and **N-carbobenzoxy-DL-alanylstearylamine** precipitated by addition of water. After thorough washing with cold dilute ammonia solution the product was crystallized from methanol; m.p. 106–109°, 8 g. *Anal.* Calcd. for $C_{29}H_{50}O_3N_2$: C, 73.37; H, 10.62; N, 5.90. Found: C, 72.88; H, 10.53; N, 6.23.

The carbobenzoxy derivative (4.7 g.) was dissolved in dry methanol (100 ml.), 0.25 g. of 10% Pd-on-carbon powder (Baker Co.) was added and the mixture stirred in an atmosphere of hydrogen. Hydrogen uptake was almost complete within a few hours, but the reaction was continued overnight. After removal of the catalyst, the solvent was removed under reduced pressure, and the residue heated at 80–90° for a few hours. The free peptide melts at 76–78° (methanol).

Anal. Calcd. for $C_{21}H_{44}ON_2$: C, 74.05; H, 13.02; N, 8.23. Found: C, 73.46; H, 12.56; N, 8.21.

L-Alanylstearylamine, m.p. 70–73° (dry ether). *Anal.* Calcd. for $C_{21}H_{44}ON_2$: C, 74.05; H, 13.02; N, 8.23. Found: C, 73.49; H, 12.77; N, 7.99. N-Carbobenzoxy derivative, m.p. 103–104° (methanol). *Anal.* Calcd. for

(21) Prepared by a slight modification of the method of Hell.[12] α-Bromostearic acid (10 g.) was heated with excess 27% aqueous ammonia (50 cc.) in a pressure bottle for 24 hr. The amino acid was collected, washed with water and then with boiling methanol and ligroin; 8.5 g., m.p. 223–224° dec., satisfactory analysis.

(22) Prepared according to *Organic Syntheses*, Coll. Vol., 3, p. 168; use of a vibromixer and extension of the reaction period to 3–4 hr. increases the yield to 80%.

$C_{29}H_{50}N_2$: C, 73.37; H, 10.62; N, 5.90. Found: C, 73.68; H, 10.64; N, 6.23.

L-Alanylcetylamine, R = $C_{16}H_{33}$, R′ = CH_3, m.p. 58–60° (ether). *Anal.* Calcd. for $C_{19}H_{40}ON_2 \cdot {}^1/_2H_2O$: C, 70.97; H, 12.85; N, 8.71. Found: C, 71.16; H, 12.30; N, 8.24. N-Carbobenzoxy derivative, m.p. 90–93° (methanol). *Anal.* Calcd. for $C_{27}H_{46}O_3N_2$: C, 72.60; H, 10.38; N, 6.27. Found: C, 72.87; H, 10.72; N, 6.14.

L-Alanyl-ω-cyclohexyl-*n*-decylamine, R = $C_{16}H_{31}$, R′ = CH_3, m.p. 56–58° (methanol). *Anal.* Calcd. for $C_{19}H_{38}ON_2$: C, 73.44; H, 12.34. Found: C, 73.46; H, 12.24. N-Carbobenzoxy derivative, m.p. 115–116° (methanol). *Anal.* Calcd. for $C_{27}H_{44}O_3N_2$: C, 72.93; H, 9.97; N, 6.30. Found: C, 73.13; H, 9.98; N, 6.19.

L-Leucylstearylamine, R = $C_{18}H_{37}$, R′ = C_5H_{10}, m.p. 66–68° (methanol). *Anal.* Calcd. for $C_{24}H_{50}ON_2$: C, 75.33; H, 13.17; N, 7.32. Found: C, 75.20; H, 12.92; N, 7.06. N-Carbobenzoxy derivative, m.p. 96–98° (methanol). *Anal.* Calcd. for $C_{32}H_{56}O_3N_2 \cdot {}^1/_2H_2O$: C, 73.09; H, 10.92; N, 5.33. Found: C, 73.12; H, 10.77; N, 5.31.

L-Leucylcetylamine, R = $C_{16}H_{33}$, R′ = C_5H_{10}, m.p. 58–60° (methanol). *Anal.* Calcd. for $C_{22}H_{46}ON_2$: C, 74.51; H, 13.08; N, 7.90. Found: C, 74.32; H, 13.08; N, 8.15. N-Carbobenzoxy derivative, m.p. 95–97° (methanol). *Anal.* Calcd. for $C_{30}H_{52}O_3N_2$: C, 73.72; H, 10.72; N, 5.73. Found: C, 74.04; H, 10.55; N, 5.89.

L-Prolylstearylamine, m.p. 70–72° (methanol). *Anal.* Calcd. for $C_{23}H_{46}ON_2$: C, 75.35; H, 12.65; N, 7.64. Found: C, 75.93; H, 12.56; N, 7.25. N-Carbobenzoxy derivative, m.p. 88–90° (methanol). *Anal.* Calcd. for $C_{31}H_{52}O_3N_2$: C, 74.35; H, 10.47; N, 5.59. Found: C, 74.01; H, 10.36; N, 5.60.

Glycylstearylamine, R = $C_{18}H_{37}$, R = H, m.p. 96–98° (methanol). *Anal.* Calcd. for $C_{20}H_{42}ON_2 \cdot {}^1/_2H_2O$: C, 71.58; H, 12.91; N, 8.35. Found: C, 71.98; H, 12.79; N, 8.58. N-Carbobenzoxy derivative, m.p. 116–118° (tetrahydrofuran). *Anal.* Calcd. for $C_{28}H_{48}O_3N_2$: C, 73.00; H, 10.50; N, 6.08. Found: C, 72.81; H, 10.26; N, 6.04.

Glycylcetylamine, R = $C_{13}H_{33}$, R′ = H, m.p. 84–86° (methanol). *Anal.* Calcd. for $C_{18}H_{38}ON_2$: C, 72.42; H, 12.83; N, 9.39. Found: C, 72.19; H, 12.47; N, 9.39. N-Carbobenzoxy derivative, m.p. 110–111° (methanol). *Anal.* Calcd. for $C_{26}H_{44}O_3N_2$: C, 72.18; H, 10.25; N, 6.48. Found: C, 71.80; H, 10.25; N, 6.44.

β-Alanylstearylamine, $C_{18}H_{37}NHCOCH_2CH_2NH_2$, m.p. 85–87°. *Anal.* Calcd. for $C_{21}H_{44}ON_2 \cdot {}^1/_2H_2O$: C, 72.14; H, 12.97; N, 8.01. Found: C, 72.37; H, 12.99; N, 7.99. N-Carbobenzoxy derivative, m.p. 124–126° (tetrahydrofuran–methanol). *Anal.* Calcd. for $C_{29}H_{50}O_3N_2$: C, 73.37; H, 10.62; N, 5.92. Found: C, 73.24; H, 10.57; N, 5.50. Carbamate, m.p. 126–127° (methanol). *Anal.* Calcd. for $C_{43}H_{88}O_4N_2$: C, 71.21; H, 12.23; N, 7.72. Found: C, 71.72; H, 12.36; N, 7.96.

β-Alanylcetylamine, $C_{16}H_{33}NHCOCH_2CH_2NH_2$, m.p. 84–86° (ether). *Anal.* Calcd. for $C_{19}H_{40}ON_2 \cdot {}^1/_2H_2O$: C, 70.98; H, 12.85; N, 8.71. Found: C, 70.73; H, 12.89; N, 8.73. N-Carbobenzoxy derivative, m.p. 124–126° (dioxane–methanol). *Anal.* Calcd. for $C_{27}H_{46}O_3N_2$: C, 72.60; H, 10.38; N, 6.27. Found: C, 72.20; H, 10.23; N, 6.30. Carbamate, m.p. 112–114° (methanol). *Anal.* Calcd. for $C_{39}H_{80}O_4N_2$: C, 70.01; H, 12.05; N, 8.37. Found: C, 69.63; H, 12.03; N, 8.05.

N-Cysteinylstearylamine, $C_{18}H_{37}NHCOCH(SH)NH_2$.—This substance was obtained by sodium–liquid ammonia reduction of N-carbobenzoxy-L-cystinylstearylamine, $[C_6H_5CH_2OCONHCH(CONHC_{18}H_{37})S-]_2$, m.p. 156–161° (tetrahydrofuran). *Anal.* Calcd. for $[C_{28}H_{47}O_2N_2S]_2$: C, 68.26; H, 9.63; N, 5.69. Found: C, 68.66; H, 9.66; N, 5.47. The reduction procedure is based on that of Sifferd and du Vigneaud[23] and the crude product was obtained in 40% yield. Repeated crystallizations gave a product with a constant m.p. of 74–76°, but this was not analytically pure.

N-Stearyl-L-asparagine, $C_{18}H_{37}NHCOCH_2CH(NH_2)COOH$.–N-Carbobenzoxyaspartic acid anhydride[24] (7.56 g.) in benzyl alcohol (35 ml.) was cooled to ice-bath temperature and one equiv. of sodium benzylate[25] was added. After one hour, the α-benzyl ester, $C_6H_5CH_2OCONHCH(OCOCH_2C_6H_5)CH_2COOH$ (7 g.), was isolated and purified according to the literature method.[26] The half-ester was condensed with stearylamine *via* the mixed anhydride with ethyl chlorocarbonate to give the dicarbobenzoxy derivative of stearyl-L-asparagine, m.p. 92–94° (methanol). Hydrogenolysis of the carbobenzoxy groups (methanol, Pd on charcoal), gives N-stearyl-L-asparagine in 60% yield, m.p. 168–170° (methanol).

Anal. Calcd. for $C_{22}H_{44}O_3N_2 \cdot H_2O$: C, 65.63; H, 11.52. Found: C, 65.68; H, 11.24.

Miscellaneous Tripeptides.—**L-Alanyl-L-alanylstearylamine**, $C_{18}H_{37}NHCOCH(CH_3)NHCOCH(CH_3)NH_2$, was prepared by condensation of N-carbobenzoxy-L-alanine with L-alanylstearylamine followed by hydrogenolysis of the protective group (80% over-all yield), m.p. 115–117° (methanol). *Anal.* Calcd. for $C_{24}H_{49}O_2N_3$: C, 70.02; H, 12.00; N, 10.21. Found: C, 70.06; H, 12.38. N, 10.12. Carbobenzoxy derivative, m.p. 163–164° (tetrahydrofuran and methanol). *Anal.* Calcd. for $C_{32}H_{55}O_4N_3$: C, 70.42; H, 10.16; N, 7.70. Found: C, 70.12; H, 10.27; N, 7.48.

β-Alanyl-β-alanylstearylamine, $C_{18}H_{37}NHCOCH_2CH_2NHCOCH_2CH_2NH_2$, was prepared by above procedure; m.p. 160–163°. *Anal.* Calcd. for $C_{24}H_{49}O_2N_3 \cdot H_2O$: C, 67.09; H, 11.96; N, 9.78. Found: C, 66.99; H, 11.67; N, 9.27. Carbobenzoxy derivative, m.p. 175–178°.

Ethylene glycol monostearyl ether (Dr. Wei-Yuan Huang and S. B.) was prepared by a different procedure from that reported in the literature.[27] A mixture of ethylene glycol (84 ml.), sodium (1.5 g.), octadecyl bromide (20 g.) and tetrahydrofuran (10 ml.) was heated at 120° for 96 hr. The mixture was then cooled, diluted with water and extracted with ether. The extracted material after removal of the solvent was crystallized from dry acetone. The first crystallizate (4.3 g., m.p. 55–57°) is probably impure ethylene glycol distearyl ether (lit. m.p. 56.5–57°).[27] On concentration of the mother liquors the monostearyl ether separates as a white flaky solid, 11.7 g., m.p. 51–52° (lit.[27] 51.5–52.5°).

An attempted preparation (E. T.) of this monoether by lithium aluminum hydride reduction of the **stearyl ether of β-hydroxypropionic acid**, $C_{18}H_{37}OCH_2CH_2COOH$, or the ester was not successful because the desired product was contaminated with stearyl alcohol, formed by reductive cleavage of the ether bond. The acid was prepared by the addition of stearyl alcohol (27 g.) to methyl acrylate (13 g., 50% excess) in dry dioxane solution containing a trace of piperidine and trimethylbenzylammonium hydroxide. The mixture was refluxed overnight and concentrated. The crude product was precipitated with water, and after washing with water was refluxed in a dilute alkaline solution (8 g. of KOH in 500 ml. of H_2O). Unchanged stearyl alcohol was removed by filtration and the product precipitated by addition of acid. It was taken into ether and then precipitated as the ammonium salt with gaseous ammonia. The free acid was obtained by acidification of an aqueous solution of the salt, m.p. 75–78° (ether), 4.5 g. (13%).

Anal. Calcd. for $C_{21}H_{42}O_3$: C, 73.63; H, 12.36. Found: C, 73.34; H, 12.26.

The methyl ester was prepared through the mixed anhydride with chlorocarbonic acid. The acid (1.7 g.) was dissolved in dry tetrahydrofuran containing a trace of triethylamine; the solution was cooled to 0° and ethyl chlorocarbonate (0.5 ml.) was added. After a few minutes dry methanol was added, and the stirred mixture allowed to come to room temperature. The product was purified in the usual way; 1.7 g., m.p. 53–56° (methanol).

Anal. Calcd. for $C_{22}H_{44}O_3$: C, 74.10; H, 12.44. Found: C, 74.39; H, 12.75.

The amide was prepared by the same procedure (concd. aqueous ammonia); m.p. 95–97° from tetrahydrofuran–ether.

(23) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

(24) Prepared by the procedure used for the preparation of N-carbobenzoxyglutamic acid anhydride, V. du Vigneaud and G. L. Miller, *Biochem. Prep.*, **3**, 79 (1952).

(25) Prepared by dissolving 0.46 g. of sodium in methanol, then adding benzyl alcohol and finally removing the methanol by distillation.

(26) M. Bergmann, L. Zervas and L. Salzmann, *Ber.*, **66**, 1288 (1933). These investigators carried out the condensation in a sealed tube at an elevated temperature (no basic catalyst).

(27) D. A. Shirley, J. R. Zietz, Jr., and W. H. Reedy, *J. Org. Chem.*, **18**, 378 (1953).

Anal. Calcd. for $C_{21}H_{43}ON$: C, 73.84; H, 12.09; N, 4.10. Found: C, 73.70; H, 12.60; N, 3.90.

Typical Emulsion Experiments.—The substances under test were dissolved in 20 ml. of water. The maximum concentration possible was generally employed since none were markedly soluble. Satisfactory results were obtained only when the solution contained 0.10–0.20 g. of emulsifier of the type investigated. This solution was mixed in an Omnimixer with 5 ml. of Nujol containing 0.2 g. of cholesterol. The emulsions were judged on the basis of stability and of particle size; in our experiments only emulsions of small particle size (1–2 μ) exhibited appreciable stability.

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Investigations on Lignin and Lignification. XVI. On the Mechanism of the Biogenesis of Methyl *p*-Methoxycinnamate and Its Possible Relation to Lignification[1]

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The mold *Lentinus lepideus* is known to produce methyl *p*-methoxycinnamate when grown on carbohydrate or ethyl alcohol as the sole carbon source. A number of metabolic products have been found and identified in the culture medium of this mold. These are 5 ketoacids, 3 sugars and shikimic acid. The results point to a relationship between the biogenesis of methyl *p*-methoxycinnamate and of tyrosine. A possible relation to the formation of lignin building stones is discussed.

Among the several varieties of wood-destroying molds, *Lentinus lepideus* (Lelep) is known to produce the so-called brown-rot in wood. It has been found that the metabolic processes associated with the decay of wood by this organism give rise to aromatic substances. These are methyl cinnamate, methyl anisate and, particularly, methyl *p*-methoxycinnamate.[2]

Studies in this Laboratory now have shown that it is possible to grow Lelep on media containing glucose, xylose or ethyl alcohol as sole carbon source, whereby methyl *p*-methoxycinnamate (I) appears as a crystalline deposit in the culture medium, after several weeks of incubation.[3] From these results it was concluded that (I) is not a product of the degradation of lignin, which might conceivably have been effected by the organism during its growth on wood. For, starting from carbohydrates or ethyl alcohol, Lelep is quite capable of synthesizing the above aromatic compound I.

Furthermore, Lelep possesses the remarkable property of being able to detoxify phenolic compounds by methylation. Therefore, we may feel quite certain that (I) is formed by methylation of *p*-hydroxycinnamic acid (Ia), an assumption which will receive support from an additional fact to be discussed later.

Recent reports seem to confirm[4] the hypothesis that hydroxy- and methoxycinnamyl alcohols, such as coniferyl alcohol (III) and sinapyl alcohol (IV), may be considered as building stones of lignin. On the other hand, from certain types of lignin, *p*-hydroxybenzaldehyde,[5] as well as vanillin and syringaldehyde, were detected as products of a mild alkaline oxidation. This would indicate that *p*-hydroxycinnamyl alcohol (II) has to be considered as a lignin building stone in certain plants. *p*-Hydroxycinnamyl alcohol differs from compound Ia only in the state of oxidation of the side chain, while coniferyl alcohol and sinapyl alcohol differ from it only by the presence of one or two additional methoxyl groups, respectively.

The investigation of the biogenesis of lignin building stones in plants meets with considerable experimental difficulty, due to the complex nature of the problem. However, studies of the biosynthesis of I by Lelep are somewhat simpler. Thus any results obtained from such experiments could have importance in theorizing on the formation of lignin building stones, if we assume similar pathways in the formation of both compounds.

This assumption of the similarity of the biogenesis of I with the formation of lignin building stones is based on the close structural relationship between the two compounds. The experiments described here were therefore undertaken as part of the investigation of the details of the biogenesis of lignin building stones.

The fact that in its natural state Lelep grows on wood and that, as a result of its metabolic activity, it forms a substance structurally similar to the lignin building stones, emphasizes this relationship.

Experimental

In the following experiments, Lelep was grown on 50-ml. portions of a nutrient medium contained in 125-ml. erlenmeyer flasks, under aseptic conditions. The medium was of the following composition: glucose, 15–20 g., or ethyl alcohol, 10 ml.; KH_2PO_4, 1.5 g.; Neopeptone (Difco), 1.0 g.; $MgSO_4 \cdot 7H_2O$, 0.5 g.; thiamine hydrochloride, 2.0 mg.; water, to 1.0 liter.

The addition of 4.0 g. of sodium acetate to the glucose medium had the effect of accelerating the growth and also the appearance of methyl *p*-methoxycinnamate (I). (Lelep grows rather poorly on acetic acid–sodium acetate as the

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(2) K. St. G. Cartwright and W. P. K. Findlay: "Decay of Timber and its Prevention," London, H. M. Stationery Off., 1946, p. 153. G. De Stevens and F. F. Nord, in K. Paech and M. V. Tracey, "Modern Methods of Plant Analysis," Vol. 3, Berlin, Springer Verlag, 1955, p. 392.

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