amides, activities were about one-half those of the analogous piperidino amides, while maximum activity was approached at a longer chain length than in the latter series.

These bis-amides also are powerful antagonists to the block of neuromuscular transmission produced by *d*-tubocurarine chloride, and rapidly reverse the paralysis induced by the latter. Roughly similar relationships between structure and activity, in the bis-amides, hold for the two principal kinds of activity, potentiation of diacetylcholine and antagonism of *d*-tubocurarine.

A detailed pharmacological report will be published elsewhere.

Acknowledgment.—The author is happy to express his appreciation to S. W. Blackman for the microanalyses and to Dr. E. J. deBeer, J. C. Castillo, R. V. Fanelli and A. L. Wnuck who kindly furnished the pharmacological results.

Experimental

Bis-aminoalkyl Amides.—A mixture of 1 mole of dicarboxylic ester and 2.5 moles of piperidinoethylamine was refluxed in a metal-bath at 190° for a period of from four to 24 hours. Excess amine and alcohol, formed in the reaction, were removed *in vacuo*. Upon cooling the product usually crystallized. Purification was accomplished by recrystallization from ethyl acetate or methanol–ethyl acetate mixtures. Yields were between 60 and 90%.

Bis-methiodides.—Brief refluxing of a methanol solution of the bis-β-tertiary amino amides with excess of methyl iodide usually gave nearly quantitative yields of the bis-methiodides. These were purified by recrystallization from methanol or methanol–ethyl acetate mixtures.

The above outline illustrates the preparative methods used. Details for all compounds appear in Table I. All melting points are uncorrected.

TUCKAHOE 7, NEW YORK

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA AND THE RADIATION LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

The Regeneration of Squalene from its Solid Hexahydrochloride

BY WILLIAM G. DAUBEN, H. LEON BRADLOW, N. K. FREEMAN, DAVID KRITCHEVSKY AND MARTHA KIRK

RECEIVED MARCH 10, 1952

It has been found that natural squalene is of a homogeneous trialkylethylene bond structure but that squalene which has been regenerated from its solid hexahydrochloride possesses both the trialkylethylene and the unsymmetrical dialkylethylene types of bond structures. It is estimated that between 20-40% of this latter type of structure can be present. Methyl group determination by oxidation has been shown to be unreliable. It has been found that squalene can be chromatographed on "Quilon" treated paper and can be separated from cholesterol by this method.

The recent interest[1,2] in the early postulate of Robinson[3] that the triterpene squalene could act as a precursor of cholesterol has prompted us to initiate the preparation of the compound labeled with C^{14}. Before this could be done, it has been necessary to reinvestigate certain phases of the chemistry of this hydrocarbon. Heilbron and his collaborators[4] have studied, in detail, the isolation and purification of squalene. They found that the solid hexahydrochloride which is easily prepared and purified is a convenient intermediate to employ in the purification of the compound. They reported that at least two isomeric hexahydrochlorides could be obtained but upon removal of the acid both solids yielded what was apparently the same compound. Since it has been demonstrated by various investigators[5,6] that many natural terpenes occur as a mixture of carbon–carbon double bond isomers of the isopropylidene and isopropenyl type, it was deemed important to investigate this problem with regard to natural and regenerated squalene.

Natural squalene, obtained from a commercial source,[7] was redistilled and converted into its hexahydrochloride and then regenerated by heating with pyridine as described by Heilbron.[4] The infrared spectra of the materials are shown in Fig. 1.

The infrared spectrum of squalene has been published previously[8] but no mention of the source of the material was given. The results in Fig. 1 are consistent with the accepted structure of squalene, in which all of the double bonds can be regarded as trialkylethylenes, *viz.*, $RR'C{=}CHR''$. A characteristic absorption band for this class of alkenes is found in the range from 12.0 to 12.5 μ.[5,9,10] In light of the recent spectroscopic investigation of acyclic terpenes by Barnard and his collaborators[6] it is clear that the fraction of double bonds having the alternate configuration $RR'C{=}CH_2$, is quite small and may well be zero. The characteristic band for this latter structure is more sharply defined at about 11.25μ[5,9,10] and according to Barnard, *et al.*,[6] the molar extinction coefficient of this band is four to five times as great as that of the 12μ band of the trialkylethylenes. Using this estimated ratio of intrinsic intensities, an upper limit of about 3% was set by them on the amount of $RR'C{=}CH_2$ in purified natural terpenes. Squalene conforms with this result.

The spectrum of squalene regenerated from the hexahydrochloride differs from that of natural material. A strong new absorption band appears

(1) K. Bloch, "Recent Progress in Hormone Research," Vol. VI, Academic Press, Inc., New York 10, N. Y., 1951, p. 111 ff.

(2) "Ciba Foundation Conference on Isotopes in Biochemistry," J. and A. Churchill, Ltd., London, W.1, England, p. 24 ff.

(3) R. Robinson, *J. Soc. Chem. Ind. (London)*, **53**, 1062 (1934).

(4) I. M. Heilbron, E. D. Kamm and W. M. Owens, *J. Chem. Soc.*, 1631 (1926).

(5) H. W. Thompson and D. H. Whiffen, *ibid.*, 1412 (1948).

(6) D. Barnard, L. Bateman, A. J. Harding, H. P. Koch, N. Sheppard and G. B. B. M. Sutherland, *ibid.*, 915 (1950).

(7) Technical Squalene, Control No. Q-511, purchased from Distillation Products, Inc., Rochester, New York.

(8) H. W. Thompson and P. Torkington, *Trans. Faraday Soc.*, **41**, 246 (1945).

(9) R. S. Rasmussen and R. R. Brattain, *J. Chem. Phys.*, **15**, 120 (1947).

(10) N. Sheppard and G. B. B. M. Sutherland, *Proc. Royal Soc. (London)*, **A196**, 195 (1949).

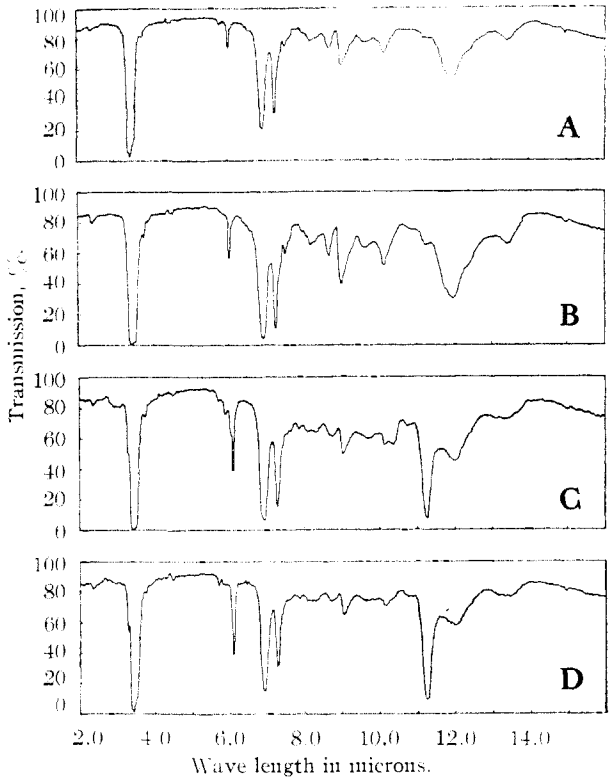


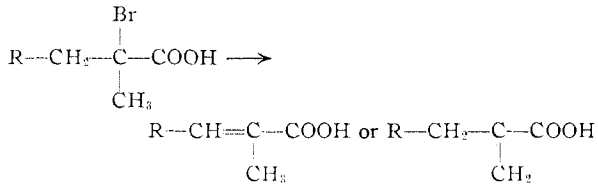
Fig. 1.—The spectra of: A, commercial squalene; B, distilled squalene; C, squalene regenerated by pyridine method; D, squalene regenerated by KO-*t*-butyl method.

at 11.25μ which points to the existence of a substantial amount of the unsymmetrical dialkylethylene structure. This is corroborated to some extent by observations in two other spectral regions. First, for natural squalene there is only one double bond stretching frequency. It appears at 6.01μ and is at the correct position for a trialkylethylene.[6] In the spectrum of regenerated squalene an additional (and stronger) band appears at 6.07μ, and this corresponds to the observed absorption of compounds containing terminal methylene groups.[6] The second point of confirmation is found in an apparent change in the number of methyl groups on regeneration. In hydrocarbons generally the absorption band at 7.25μ has been attributed to a methyl group vibration[11] and in some instances its intensity has been correlated with the number of methyl groups.[12,13] If spectra of the two substances are taken in carbon tetrachloride solution at identical concentrations and path lengths, the 7.25μ band of natural squalene is found to be stronger than in the regenerated material.

The information available is not sufficient to make a reliable quantitative estimate of the relative amounts of the two structural types in the regenerated squalene. If the ratio of the extinction coefficients for the 11.25 and 12.0μ is taken as five,[6] a value of 44% of $RR'C{=}CH_2$ is obtained. If the proportionality is assumed between the number of methyl groups and the intensity of the 7.25μ band, the amount of the terminal methylene grouping is only about 20%. Either of these assumptions, and the values obtained through them, are open to criticism.

The use of oxidative degradation as an unequivocal method for the investigation of the relative amounts of allylic isomers has become increasingly suspected in recent years.[6] Nevertheless, Kuhn–Roth C-methyl determinations were performed on all of the squalene samples in order to further evaluate the method. It was found that no great difference was noticeable; the commercial and the redistilled commercial squalene showed 64.5 and 63.5%, respectively, of six methyl groups and the regenerated sample has a value of 57.5%.[14] Although this latter value is lower, as would be expected from the above spectroscopic results, there is not a great difference. In view of the low value of natural squalene, it must be assumed that under the conditions of the oxidation, bond migration does occur.

Cason[15] has recently found that in the elimination of hydrogen bromide from α-bromo-α-methyl acids, the product obtained varies with the agent



employed to remove the acid moiety. When the elimination was conducted with potassium *t*-butoxide in *t*-butanol, it was found that a terminal methylene type of structure was generated, whereas when quinoline was used the trialkylethylene type of bond was received. When squalene hexahydrochloride was allowed to react with potassium *t*-butoxide in *t*-butanol, the squalene obtained had an identical infrared spectrum to that of the material obtained from the pyridine treatment but that the C-methyl value was 62.5% as compared to 57.5%. It would seem that no great difference exists in these products.

Since in the planned biological experiments, a convenient method of analysis for squalene and cholesterol would be needed, the chromatography of these substances on Whatman No. 1 filter paper impregnated with "Quilon" (stearato chromic chloride, du Pont)[16] was investigated. The results obtained employing methanol as the developing solvent are summarized in Table I.

TABLE I

R_f VALUES

Commercial squalene	0.71	
Distilled squalene	.71	
Regenerated squalene (pyridine)	.71	0.86
Regenerated squalene (butoxide)	.71	.86
Cholesterol	.61	

(11) R. S. Rasmussen, *J. Chem. Phys.*, **16**, 712 (1948).
(12) S. A. Francis, *ibid.*, **18**, 861 (1950).
(13) N. K. Freeman, to be published.
(14) On saturated compounds, our laboratory routinely obtains values which are around 80% of theory. With commercial farnesol, however, again only a value of 62.5% of three methyl groups was obtained.
(15) J. Cason, private communication.
(16) D. Kritchevsky and M. Calvin, THIS JOURNAL, **72**, 4330 (1950).

When only squalene was placed on the paper, it was found that both the commercial and the redistilled commercial materials gave only one spot as detected with iodine vapor and the average R_f value was 0.71. With the regenerated squalenes, however, two zones were noted, one intense at 0.71 and a weak spot at 0.86. It is interesting to note that the appearance of the two zones occurs only with the regenerated samples which also show the terminal methylene group in the infrared spectra. Such a relationship suggests that a separation has been achieved between moieties having different double bond distributions. It is also possible that a separation of *cis–trans* isomers has been performed but in view of the methods of regeneration it would be expected that triply substituted double bonds would be of the *trans* configuration. Thus, one cannot at this time allocate an exact definition to this separation of two zones on "Quilon" paper and more work must be performed along these lines.

Under these same conditions, it has been reported that cholesterol shows a R_f value of 0.56.[17] It has been found that when a mixture of cholesterol and squalene are run together, a separation of these two compounds can be achieved. For analysis, squalene was first detected with iodine vapors and then the cholesterol with silicotungstic acid.[17] The value for squalene remained as reported above but it was noted that the average R_f of cholesterol was somewhat higher than found for cholesterol alone, the value being 0.61 as compared to 0.56. This change in R_f may be due to the solvent action of squalene. Thus, the difference in the R_f values between these two spots suggest that such a method of analysis can be employed to separate squalene and cholesterol and when such a method is coupled with radioautography, a semi-quantitative analysis can be performed.

(17) D. Kritchevsky and M. R. Kirk, *Arch. Biochem. Biophys.*, **35**, 346 (1952).

Experimental

The commercial squalene was redistilled, b.p. 213° (1 mm.), $n^{20}D$ 1.4962 (lit.[4] 1.4965) and then converted into its hexahydrochloride as described by Heilbron,[4] m.p. 103–110°. The solid derivative was reconverted to squalene following the published procedure[4] and then redistilled. For regeneration from potassium *t*-butoxide, the hexahydrochloride (4.8 g.) was allowed to react with a solution of 1.4 g. of potassium in 30 ml. of dry *t*-butanol. After heating for three hours at 100°, the reaction mixture was diluted to 200 ml. with water, extracted with ether, washed, dried and distilled, yield 2.15 g. (68%).

All infrared spectra were taken as a film with an I.R. Spectrophotometer manufactured by Baird Associates.

For the chromatography, the papers used were 1.5 × 15 inch strips of Whatman No. 1 filter paper impregnated with "Quilon" (stearato chromic chloride).[16,17] The developing solvent was methanol and in every case about 10 γ of material was placed at the origin. R_f values were measured from the farthest spot of the origin to the foremost point of the moving spot. The squalene was detected by the intense brown spots visible when the strips were suspended in iodine vapor for one minute.[17] Cholesterol was detected with a silicotungstic acid spray.[16,17] In experiments where squalene and cholesterol were run together, the squalene was detected first using the iodine vapor then, after the papers had been kept in a well-ventilated hood overnight to ensure complete evaporation of the iodine, the cholesterol was located with silicotungstic acid. All R_f values reported are an average value of at least twelve separate chromatograms and the standard deviation was ± 0.01 R_f unit for squalene run alone and ± 0.02 R_f unit for squalene and cholesterol run together.

BERKELEY, CALIFORNIA

[CONTRIBUTION NO. 242 FROM THE CHEMICAL DEPARTMENT, EXPERIMENTAL STATION, E. I. DU PONT DE NEMOURS & COMPANY]

Telomerization of Vinyl Monomers with Hydrogen Chloride[1]

BY T. A. FORD, W. E. HANFORD, J. HARMON AND R. D. LIPSCOMB

RECEIVED MARCH 19, 1952

Ethylene reacts with hydrogen chloride in the presence of free radical initiators and water to yield mixtures of straight-chain primary alkyl chlorides having even numbers of carbon atoms. Other polymerizable unsaturated compounds appear to undergo similar reactions, but the structures of the products have not been established.

The free radical-initiated reaction of halomethanes with olefins yields addition products derived from one or more molecules of olefin per molecule of halomethane.[2–4] For example, ethylene and carbon tetrachloride yield a series of $\alpha,\alpha,\alpha,\omega$-tetrachloroalkanes.[3] This paper is concerned with a further example of this type of reaction in which hydrogen chloride acts as the telogen.[5]

Reaction of ethylene with hydrogen chloride in the presence of free radical-forming initiators has been found to yield mixtures of straight-chain primary alkyl chlorides having even numbers of carbon atoms and ranging from ethyl chloride *n*-butyl chloride, *n*-hexyl chloride, etc., to greases and waxes having chain lengths of 40 or more carbon atoms.[6] Other polymerizable olefinic compounds, such as styrene and allyl chloride, can also be telomerized with hydrogen chloride.[7]

(1) Presented in part at the 113th Meeting of the American Chemical Society, Chicago, Ill., April, 1948.

(2) M. S. Kharasch, E. V. Jensen and W. H. Urry, THIS JOURNAL, **69**, 1100 (1947).

(3) R. M. Joyce, W. E. Hanford and J. Harmon, *ibid.*, **70**, 2529 (1948).

(4) J. Harmon, T. A. Ford, W. E. Hanford and R. M. Joyce, *ibid.*, **72**, 2213 (1950).

(5) These reactions represent a type of polymerization, and the process by which the end groups are formed is sometimes referred to as chain transfer. For reactions of this sort that lead to short chain compounds in which the end groups are chemically significant, rather than to high polymers in which the end groups are relatively insignificant, we have found it convenient to refer to the reaction as telomerization, to the products of the reaction as telomers, and to the molecule which gives rise to the end groups as the telogen (derived from the Greek roots "telos" meaning "end," and "gen" meaning "former").

(6) W. E. Hanford and J. Harmon, U. S. Patent 2,418,832 (April 15, 1947).

(7) W. E. Hanford and J. Harmon, U. S. Patent 2,440,801 (May 4, 1948).