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ISOLATION OF ISOPRENOID ACIDS FROM A CALIFORNIA PETROLEUM[1]

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Abstract—By sequences involving fractional distillation, thiourea adduction, gas chromatography and crystallization of solid derivatives, there have been isolated from the naphthenic acids of a California petroleum four acyclic acids of isoprenoid structure: 2,6,10-trimethylhendecanoic acid, 3,7,11-trimethyldodecanoic acid, 2,6,10,14-tetramethylpentadecanoic acid, 3,7,11,15-tetramethylhexadecanoic acid. Structures were deduced by interpretation of spectra and confirmed by comparison with samples of the synthetic acids. Starting material for synthesis of the C_{14} acid was farnesol, while phytol was starting material for synthesis of the C_{19} and C_{20} acids. No optical rotation could be observed for the acids isolated from petroleum. In mass spectrometry of amides of these acids, in addition to the set of fragments containing the amido grouping, there was also observed a complete set of ions containing the nitrile grouping.

INTEREST in the origin of petroleum, as may be deduced from constituents thereof, has been stimulated by recent reports[3-5] of the isolation of isoprenoid hydrocarbons therefrom. Although there has appeared a proposal that the aliphatic carboxylic acids are logical precursors[6] for the hydrocarbons found in petroleum, essentially nothing is known of the structures of the naphthenic acids with more than ten carbons.[7] Only a few of the normal isomers have been isolated and characterized. The complexity of the naphthenic acid mixture is such that separation of the components of higher molecular weight has not been possible.

Our efforts to separate pure components from the naphthenic acids have included essentially all the methods currently available, but the effective combinations involved fractional distillation, urea and thiourea adduction, gas chromatography and crystallization of solid derivatives. The present report is concerned with the isolation and characterization of four acyclic acids of the isoprenoid structure: 2,6,10-trimethylhendecanoic acid (C_{14}), 3,7,11-trimethyldodecanoic acid (C_{15}), 2,6,10,14-tetramethylpentadecanoic acid (C_{19}) and 3,7,11,15-tetramethylhexadecanoic acid (C_{20}). Structures were initially assessed by interpretation of spectra, and they were established by comparison with synthetic samples. Successful methods for separation of these acids may be illustrated by consideration of the procedures used for securing the C_{15} acid.

[1] This investigation was supported by a grant from the Petroleum Research Fund of the American Chemical Society.

[2] Recipient of a Procter and Gamble Co. pre-doctoral Research Fellowship, 1963, 64.

[3] R. A. Dean and E. V. Whitehead, *Tetrahedron Letters* No. 21, 768 (1961).

[4] J. G. Bendoraitis, B. L. Brown and L. S. Hepner, *Analyt. Chem.* **34**, 49 (1962).

[5] J. J. Cummins and W. E. Robinson, *J. Chem. Eng. Data* **9**, 304 (1964).

[6] J. E. Cooper and E. E. Bray, *Geochim. et Cosmochim. Acta* **27**, 1113 (1963).

[7] The earlier work on naphthenic acids, as well as the modern work at the University of Texas has been reviewed by H. L. Lochte and E. R. Littman, *The Petroleum Acids and Bases*. Chemical Publishing, New York (1955).

These separations also illustrate the remarkable complexity of the naphthenic acid mixture.

The acids used for this investigation were from middle distillates of a San Joaquin Valley naphthenic type crude.[8] For subsequent separation of components, it proved most convenient to distil the methyl esters through a half-meter Podielniak-type column and to collect fractions such that two of them were of b.p. 135–147°/10 mm

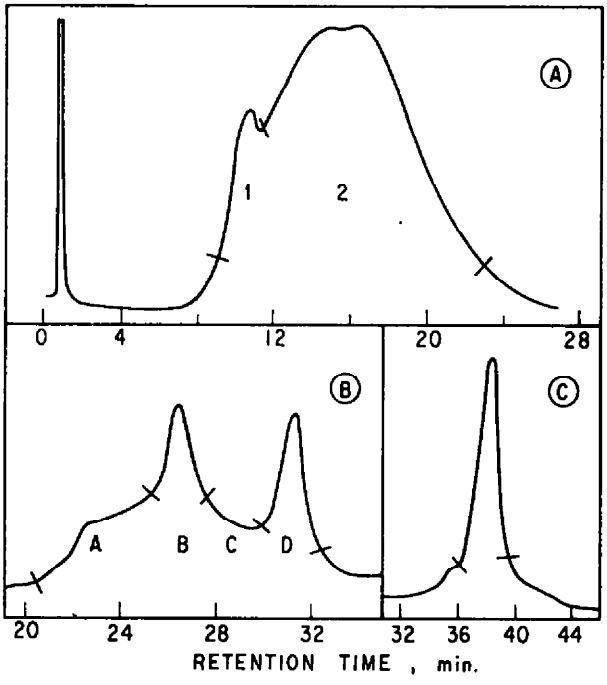


FIG. 1. Tracings from gas chromatography of methyl naphthenates; fractions cut at cross lines.

Tracing A: 200 μl sample, b.p. 147–156°/10 mm; 2·44 m × 1·9 cm column, 10% NPGS on Chromosorb P; temp 200°; He flow rate 250 ml/min.

Tracing B: 15 μl of Cut 1, Tracing A; 6 m × 0·95 cm column; 10% GE-SF-96 on Chromosorb P; temp 177°; He flow rate 150 ml/min.

Tracing C: 4 μl of Cut D, Tracing B; 6 m × 0·95 cm column, 10% NPGS on Chromosorb P; temp 170°; He flow rate 150 ml/min.

and 147–156°/10 mm. Gas chromatography of each of these fractions on neopentylglycol succinate (NPGS) gave a broad, largely unresolved band of 20-25 minutes in width; however, one peak in each tracing projected slightly above the continuum. In the tracing from the higher-boiling fraction (Fig. 1, Tracing A), this peak was near the beginning of the broad band, and this is the fraction from which it proved possible to isolate a relatively homogeneous component by gas chromatography. This distillation cut was about 10% of the naphthenic acid fraction employed. Although rechromatography of Cut 1, Tracing A, on the same partitioning agent (NPGS) gave a single symmetrical band, chromatography on silicone (Tracing B) revealed the presence

[8] These acids were supplied by the Standard Oil Co. of California, through the generous assistance of Dr. A. H. Batchelder of the California Research Corp.

of at least six components. Cut B from this chromatography (2% of the distillation fraction) proved to be methyl tridecanoate, while Cut D (also about 2%) consisted largely of the C_{15} isoprenoid acid. It is of interest that a third chromatography of Cut D (Tracing C) on the partitioning agent originally used (NPGS) permitted elimination of at least two minor impurities.[9] Still a fourth chromatography on silicone eliminated a small leading band similar to that shown in Tracing C. Mass spectra (see below)

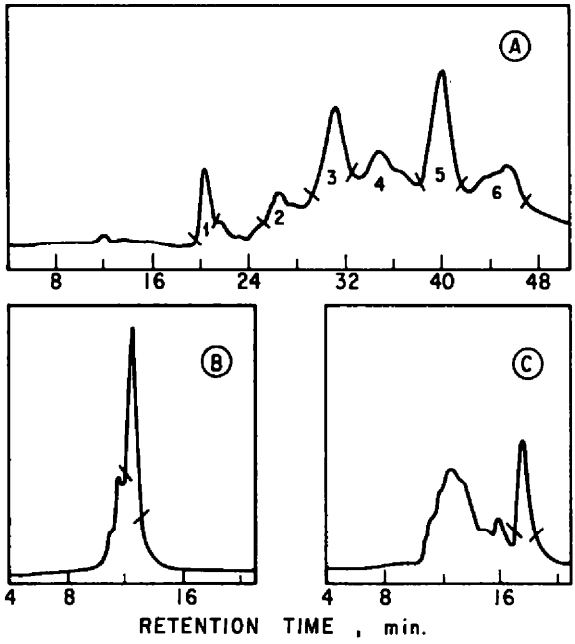


FIG. 2. Gas chromatography of methyl naphthenates; fractions cut at cross lines.

Tracing A: 20 μl sample from product obtained after two adductions with thiourea of ester of b.p. 135–147°/10 mm; 6 m × 0·95 cm column, 10% NPGS on Chromosorb P; temp 203°; He flow rate 150 ml/min.

Tracing B: 2 μl sample of Cut 1, Tracing A; 6 m × 0·95 cm column, 10% GE-SF-96 silicone on Chromosorb P; temp 172°; He flow rate 165 ml/min.

Tracing C: 2 μl sample of Cut 3, Tracing A; same column and conditions as Tracing B.

subsequently showed that this fourth chromatography did improve the purity of the ester; however, a still better sample of this ester was obtained by inclusion of thiourea adduction in the purification scheme.

Although it was not possible to separate a reasonably homogeneous sample of the ester of the C_{15} isoprenoid acid by gas chromatography of the distillation fraction of b.p. 135–147°/10 mm, this fraction was richer in this component than was the higher-boiling fraction. Use of thiourea adduction rendered possible separation of the esters of both the C_{14} and C_{15} isoprenoid acids, although in very low yield. In Fig. 2, Tracing A, is shown the pattern obtained from esters which had been adducted

[9] This type of behavior has been noted regularly in separation of the naphthenic esters. The difference in behavior in chromatographing Cut 1 and Cut D on NPGS is ascribed to the fact that in the case of Cut 1 the chromatography is not actually on NPGS but rather on NPGS in which are dissolved the several component naphthenates separated in the chromatography shown in Tracing B.

with thiourea, recovered, and adducted a second time. The sequence of prominent peaks in this tracing is in sharp contrast to the tracing from the whole distillation fraction (similar to Fig. 1, Tracing A). The bands of interest to the present investigation are those numbered 1 and 3. Re-chromatography of Cut 1 on silicone (Fig. 2, Tracing B) showed presence of at least three components, but the major cut was a relatively homogeneous sample of the ester of the C_{14} isoprenoid acid. Rechromatography of Cut 3 (Tracing C), showing presence of at least eight components, illustrates again the complexity of the naphthenic acid mixture, as well as the effectiveness of multiple gas chromatography. The resolved band in Tracing C was the best isolated sample of the ester of the C_{15} isoprenoid acid (cf. below and Table 1).

The NMR spectrum of the ester in Cut D, Tracing B, Fig. 1, showed presence of four methyl groups (ratio of methyl band to methoxyl band was 4·3:1[10]). A doublet representing two alpha hydrogens (centered at about τ 7·9, with the major peak the upfield one) showed absence of an alpha substituent, presence of a beta substituent. In the IR, there was observed the strong band (8·55 μ) due to skeletal vibrations of a terminal isopropyl group, the characteristic doublet (7·25, 7·30 μ) due to the CH deformation vibrations of this group, and the weak band at 13·6 μ due to the methylene rocking vibration of $-(CH_2)_3-$[11]. Absence of $-(CH_2)_4-$ was indicated since there was no shoulder at 13·8 μ.[12] The isoprenoid structure, strongly indicated by these data, is fully substantiated by the mass spectra of several samples.

Significant features[13] of the mass spectra (cf. Table 1) include: the rearrangement peak at m/e 74, indicating no alpha substituent; the base peak at m/e 101, indicating a β-methyl; the relatively high peaks at m/e 143 and 171, bracketing the low peak at m/e 157, indicating a 7-methyl substituent; the molecular ion at m/e 256, indicating the methyl ester of a C_{15} saturated acyclic acid. The fact that the M − 57 and M − 43 peaks, from expulsion of carbons-2, -3 and -4, and of -2 and -3, respectively, are larger than the M − 29 peak also supports the 3-methyl substituent. The increase in purity of the three samples of isolated ester is clearly indicated by the intensity of the m/e 157 peak. The intensity of this peak decreases as the purity is increased, but in the best sample of isolated ester it remains higher than is the case in the authentic sample. However, the near identity of the complete fragmentation patterns of the best sample of isolated ester and the synthetic sample establish the naturally occurring acid as having the C_{15} isoprenoid structure. A pure derivative of this acid was obtained by converting the methyl ester to the crystalline *p*-phthalimidophenacyl ester,[14] m.p. 99–101°, no depresssion on admixture with the derivative of the synthetic DL-acid. Retention times of the synthetic and isolated esters were identical on both NPGS

[10] In the examination of the NMR spectra of the methyl esters of various branched-chain acids, it has been found [J. Cason and G. L. Lange, *J. Org. Chem.* **29**, 2107 (1964)] that, in acids with three or more methyl substituents, the area of the extrapolated methyl band is significantly larger than is attributable to the number of hydrogens present in methyl.

[11] H. L. McMurry and V. Thornton, *Analyt. Chem.* **24**, 318 (1952).

[12] J. Pliva and N. A. Sorensen, *Acta Chem. Scand.* **4**, 846 (1950).

[13] Of the several discussions of mass spectra of carboxylic acid esters, probably the best and most authoritative is that by R. Ryhage and E. Stenhagen, *Mass Spectrometry of Organic Ions* (Edited by F. W. McLafferty) p. 399. Academic Press, London (1963).

[14] We are indebted to Dr. F. H. Stodola, of the Northern Regional Research Laboratory, Peoria, Ill., who supplied us with information on the formation of this derivative, prior to its publication: F. H. Stodola, *Microchem. J.* **7**, 389 (1963).

TABLE 1. MASS SPECTRA OF SAMPLES OF METHYL 3,7,11-TRIMETHYLDODECANOATE

m/e	n[a]	Isolated samples[b]			Synthetic[c] sample
		I	II	III	
74	*d*	74·2	73·5	80·5	81·2
87	2	9·5	7·0	5·2	5·3
101	3	75·5[e]	100	100	100
115	4	4·2	3·0	2·1	2·0
129	5	3·8	3·4	2·8	2·5
139	*f*	3·5	3·6	3·5	3·2
143	6	5·0	5·1	5·2	5·0
157	7	1·3	1·1	0·6	0·2
171	8	4·0	5·2	5·6	5·0
185	9	0·7	0·5	0·3	0·2
191 (M − 65)		0·4	0·5	0·5	0·4
199 (M − 57)		0·7	0·9	0·6	0·5
213 (M − 43)		0·9	1·3	1·2	1·2
227 (M − 29)		0·4	0·6	0·4	0·3
241 (M − 15)		1·1	2·0	1·9	2·0
256	*g*	1·5	3·0	2·4	2·6

[a] Value of n in formula, $(CH_2)_n—CO_2CH_3$.

[b] Values tabulated are peak heights as per cent of the base peak. Sample I: collected as shown in Fig. 1, Tracing C. Sample II: from rechromatography of Sample I on silicone; main peak collected. Sample III: collected as shown in Fig. 2, Tracing C.

[c] We wish to express our appreciation to Dr. L. H. Sarett, Merck Sharp-Dohme Research Laboratories, who supplied us with a sample of synthetic DL-3,7,11-trimethyldodecanoic acid; ester was prepared with diazomethane.

[d] This is the rearrangement peak, $CH_2{=}C(OH)—OCH_3$

[e] In this fragmentation pattern, the base peak was m/e 43.

[f] This peak is 171 minus 32 (CH_3OH), a ketene fragment.

[g] The molecular ion. In samples I and II, there were very small peaks for molecular ions at 254 and 268 (esters of monocyclic C_{15} and C_{16} acids).

and silicone columns. No optical rotation could be observed for the natural acid $[\alpha]_D^{23} = 0 \pm 0{\cdot}24°$.

The ester of the C_{14} isoprenoid acid was collected as shown in Tracing B, Fig. 2 (cf. Experimental for additional isolation procedure). Its fragmentation pattern in mass spectrometry proved identical with that of a sample synthesized from perhydrofarnesol by way of ester cracking and ozonolysis. Both IR and NMR spectra were also identical. Chief analytical characteristics of the IR spectrum are the doublet at 7·26 and 7·34 μ and the strong band at 8·55 μ, features revealing the presence of the terminal isopropyl group. In the NMR, the α-methyl is clearly revealed by the doublet between the methyl and methylene bands and the highly split single hydrogen in the α-hydrogen band. Area of the methyl band (excluding the 2-methyl doublet) corresponds to 3·3 methyl groups.[10]

The mass spectrum of the methyl ester of the C_{14} isoprenoid acid revealed the structure with unusual clarity. The base peak at m/e 88 for the rearrangement

ion, $CH_3—CH=C(OH)OCH_3$, demonstrates the α-methyl, since this ion is at m/e 74 in absence of the α-substituent. The branch at the 6-position is revealed in the usual manner by the relatively high peaks at m/e 129 (4·7%) and 157 (5·3%), bracketing the low peak at 143 (0·3%). The rather high purity of the isolated samples of ester was indicated by m/e 143 peaks of only 1·0 and 0·7%. An additional indication of both the 2- and 6-methyl groups is furnished by presence of a relatively significant

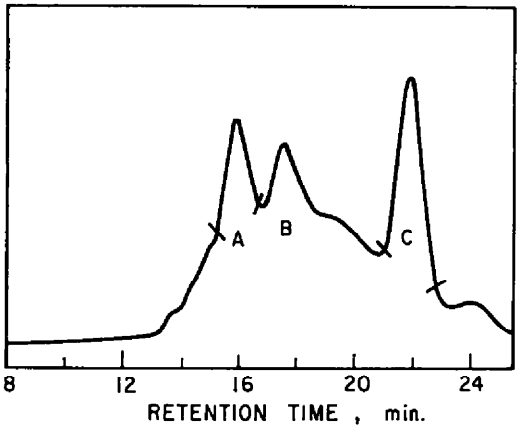


FIG. 3. Gas chromatography of methyl naphthenates, esters adducted by thiourea from fraction of b.p. 190–198°/10 mm; 6 m × 0·95 cm column; 10% GE-SF-96 on Chromosorb P; temp 210°; He flow rate 150 ml/min. Cut A is the C_{19} isoprenoid ester, Cut B is methyl heptadecanoate and Cut C is the C_{20} isoprenoid ester.

peak (3·3%) at m/e 152, which is the molecular ion (242) minus 90. It has been noted[15] in several acids containing a 6-substituent that there is a significant peak (in one instance, the base peak) at M (the molecular ion) minus the rearrangement fragment and two protons. Presumably this leaves an ion with conjugated di-unsaturation. If there is no α-substituent, this ion is at M − 76, while an α-methyl naturally gives the ion at M − 90, as observed for the C_{14} isoprenoid acid.

The C_{19} and C_{20} isoprenoid acids were isolated from the highest-boiling fraction of naphthenates, b.p. 190–198°/10 mm. This distilled fraction was 4·3% of the total esters, while the two isolated esters, in the order named, were 2·3% and 4·5% of this fraction. Of the several sequences which permitted the isolation, the most convenient utilized initial thiourea adduction of the distilled fraction, for the adducted esters represented 15% of the distilled fraction. A single chromatography of the once-adducted esters on a 6-meter silicone column (cf. Fig. 3) gave the ester of the C_{19} isoprenoid acid as a resolved band ahead of the ester of n-C_{17}, while the ester of the C_{20} isoprenoid acid was nearly midway between the esters of n-C_{17} and n-C_{18}. The complete mass spectra of the isolated isoprenoid esters or the corresponding amides were the same as those of synthetic samples. Also, the amides from the isolated and synthetic samples had the same m.p., no depression on mixing, and the *p*-phthalimidophenacyl esters of the C_{20} acids were compared.

It is of interest that the fragmentation pattern from the amides (cf. Table 2) showed a complete set of ions of the expected pattern containing the amido grouping,

[15] R. Ryhage and E. Stenhagen, *Arkiv Kemi* **15**, 333 (1960).

TABLE 2. PARTIAL MASS SPECTRA OF THE ISOLATED AND SYNTHETIC ISOPRENOID C_{20} AMIDES[a]

$(CH_2)_n$—$CONH_2$				$(CH_2)_n$—CN			
m/e	n	Isol.	Syn.	m/e	n	Isol.	Syn.
59	*b*	100%	100%	40	1	1·3%	1·4%
72	2	3·5	3·7	54	2	1·4	1·5
86	3	55·6	55·4	68	3	2·5	2·8
100	4	3·5	4·3	82	4	1·5	1·9
114	5	1·2	2·1	96	5	1·8	1·8
128	6	4·8	8·4	110	6	2·8	1·7
				111	*c*	3·2	5·0
142	7	0·3	0·4	124	7	1·9	1·3
156	8	1·6	2·7	138	8	6·5	3·8
170	9	0·5	0·8	152	9	4·1	2·5
184	10	0·2	0·4	166	10	2·4	1·4
198	11	0·4	0·7	180	11	1·3	0·8
212	12	—	0·1	194	12	0·5	0·3
226	13	0·3	0·5	208	13	6·4	3·8
240	14	0·2	0·4	222	14	0·7	0·4
254	15	0·2	0·3	236	15	0·3	0·2
268	16	0·3	0·6	250	16	0·6	0·4
282	17	—	—	264	17	—	0·1
296	18	0·7	1·3	278	18	0·6	0·3
311	*d*	0·2	0·4	293	*e*	0·1	0·1

[a] Since absolute ion intensities vary somewhat from run to run, depending on operating conditions, relative intensities of the ion peaks are the significant feature for comparison purposes.

[b] The rearrangement ion, $CH_2{=}C(OH)NH_2$.

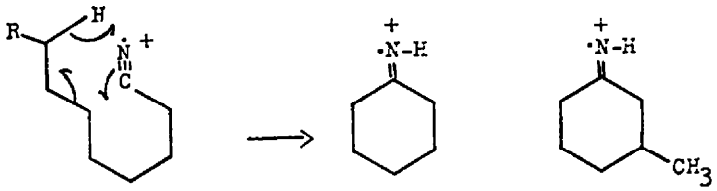
[c] The rearrangement ion from a nitrile, cf. structure II.

[d] The molecular ion of the amide.

[e] The molecular ion of the nitrile (amide—18).

and also a complete, analogous set of ions containing the nitrile grouping (amide ions minus 18). This fragmentation occurred on electron impact, not from cracking the amide in the heated inlet. This conclusion may be reached on the grounds that the inlet, at 200°, is not sufficiently hot to cause this cracking; however, characteristics of the spectrum lead to the same conclusion. It has been reported[16] that the molecular ion (M) of aliphatic nitriles is nearly always of lower intensity than the ions at M − 1 and M + 1. In contrast, the spectrum of the C_{20} amide showed peaks at m/e 292 and 294 less than half the size of the peak at 293 (M for the nitrile). The C_{19} amide showed analogous peaks. In addition, long-chain nitriles show very intense peaks (frequently the base peak) at m/e 97. This ion (I) is postulated to arise by a rearrangement of the type shown. From the C_{20} isoprenoid amide, with a β-substituent, the corresponding ion, II, would have m/e 111. As recorded in Table 2, the ion at m/e 111 is small compared to the base peak for the amide, although many of the nitrile fragments are even larger than the corresponding ones with the amide grouping. A similar effect was observed for the m/e 125 fragment for the C_{19} amide, with substituent methyls at both the 2- and 6-positions.

[16] F. W. McLafferty, *Analyt. Chem.* **34**, 26 (1962).



I II

It may be noted that the data in Table 2 reveal the positions of substituent methyl groups, in both the nitrile and amide fragments. The peak at n = 3 is very large compared to all other peaks except the rearrangment ion, the expected observation when a β-methyl is present. Branches at the 7- and 11-positions are revealed by the relatively low intensity when n = 7 and 12, with relatively high intensity on either side of these fragments. The virtual absence of a fragment when n = 17, requiring loss of two methyls, is not a reliable indication of the iso end-group. A better indication of this structural feature is the IR spectrum, as discussed in connection with the C_{15} isoprenoid acid.

The structure of the C_{19} isoprenoid acid was revealed by the IR, NMR and mass spectra of its derivatives, and the identity of these with the synthetic specimen, in a manner similar to that described for the C_{14} acid. There appeared, however, one feature of the mass spectrum which is rather unique. In normal fashion, the base peak was at m/e 88, from the rearrangement ion; the branch at the 6-position was revealed by high peaks at 129 (5·4%) and 157 (8·2%) bracketing the low peak at 143 (1·4%); and the M − 90 peak was of significant intensity (2·9%). The branch at C_{10}, however, was not revealed in normal fashion, in that the peaks normally of relatively high intensity at m/e 199 and 227 were 0·8% and 0·7%, respectively, bracketing the 213 peak of 0·7%. Since the NMR had shown the presence of four substituent methyls in the isolated ester, and the IR also indicated the isoprenoid structure, the synthetic acid was prepared, and it was found to exhibit an identical anomalous behavior in fragmentation around the C_{10} branch. Intensities observed for ions from the synthetic acid at m/e 199, 213 and 227 were 0·5, 0·3 and 0·4%, respectively. There appears to be available insufficient data to allow any generalization concerning structural features required to cause this type of anomalous fragmentation in the region of a branched chain.

The C_{19} isoprenoid acid was synthesized by hydrogenation of phytol, cracking of the acetate to the 1-alkene, and permanganate oxidation of the alkene to give the desired acid. The permanganate oxidation gave considerable cleavage (at the branches presumably), so that separation of lower molecular weight fragments by gas chromatography of the methyl ester proved necessary in order to secure a satisfactorily pure sample. The C_{19} amide, synthetic and isolated, had respective m.p. 64–66° and 63–65°, no depression on mixing.

The C_{20} isoprenoid acid was also synthesized from dihydrophytol, by chromic acid oxidation. Although this method is rather unsatisfactory, on account of obtaining the dihydrophytyl ester as product, and gives a poor yield, it was judged superior to permanganate oxidation, on account of the extensive degradation to lower molecular weight fragments which the latter reagent gave. The synthetic acid showed

the expected small optical rotation, $[\alpha]_D^{23} = -0{\cdot}17 \pm 0{\cdot}05°$, in contrast with the isolated acid, in which no optical rotation could be observed, $[\alpha]_D^{23} = 0 \pm 0{\cdot}05°$. In spite of this difference in stereochemistry, the C_{20} amides, synthetic and natural, had m.p. 51–53°, no depression on mixing. Similarly, the *p*-phthalimidophenacyl esters, synthetic and isolated, had m.p. 99–101°, no depression on mixing.

The C_{19} isoprenoid acid has very recently been reported in butterfat;[17] however, no details or properties were included. The C_{20} isoprenoid acid was first isolated from butterfat,[18] has later been reported in ox blood.[19] The structure of this acid was first reported[20] by Dutch investigators. Although the deduction of structure seems rather poorly based[21] it has been confirmed by the work of Swedish investigators[22] with long experience in the field of fatty acids. This C_{20} isoprenoid acid from butterfat was reported as having a significant specific rotation, 1·1°[18] and 1·2°.[20] As mentioned above, no optical rotation could be observed for the C_{20} isprenoid acid from petroleum, and the acid synthesized from phytol had a specific rotation of −0·17°. If phytol is the biogenetic precursor of the C_{20} isoprenoid acid from butterfat, the significant positive rotation must arise from asymmetric bio-reduction of the double bond.

Other phases of our present investigations have resulted in isolation and identification of additional isoprenoid acids, acyclic and cyclic; and the series of n-acids has been found present; however, other types of acids which have been isolated have not yet been identified.

EXPERIMENTAL

Physical measurements. IR spectra were recorded on a Perkin-Elmer Infracord, using CCl_4 solutions in matched 0·1-mm NaCl cells for solids and a thin film for liquids. NMR spectra were recorded on a Varian A-60 spectrometer, at 60 mc, with CCl_4 as solvent and tetramethylsilane as internal standard; values on the tau scale (ppm) are reported with reference to TMS at a value of 10. Mass spectra for the C_{15} compounds were determined on a C.E.C. mass spectrometer, model 21-103C, with ionizing voltage of 70 ev, and a glass inlet heated to about 200°. Some of the other spectra, including those in Table 2, were determined on this same machine after it had been equipped with

[17] R. P. Hansen, *Nature, Lond.* **201**, 192 (1964).

[18] R. P. Hansen and F. B. Shorland, *Biochem. J.* **55**, 662 (1953).

[19] A. K. Lough, *Biochem. J.* **86**, 14P (1963).

[20] W. Sonneveld, P. H. Begemann, G. J. van Beers, R. Keunig and J. C. M. Schogt, *J. Lipid Research* **3**, 351 (1962).

[21] The NMR spectrum was used to determine the number of branching methyls, by comparison of the areas of the methyl band and the methylene-methinyl band. Since these bands overlap seriously in a multibranched acid, any error in extrapolation is multiplied by two; this multiplication of the extrapolation error is avoided by comparison with the methoxyl hydrogens in a methyl ester.[10] Also, the formula used to calculate the ratio of area in the methyl to that in the methylene-methinyl band was $[3(m + 1)]/[2n - 3m - 4]$, where m is the number of branches and n is the total number of carbon atoms. This formula is in error, in that the final term in the denominator should be 6; the terminal methyl, the α-carbon and the carboxyl carbon bear no hydrogens appearing in the methylene-methinyl band. In addition, the published[20] NMR spectrum (at an unspecified frequency) was rather poor, in that there was no resolution in the methyl band, and it was overlapped by the methylene band for a full two-thirds of its height. In contrast, the NMR spectrum of our C_{20} isoprenoid acid showed resolution of several peaks in the methyl band, which was overlapped by the methylene-methinyl band to the extent of only 17% of its height. The interpretation of the mass spectral data on the isoprenoid acid from butter seemed rather esoteric, and the reference cited as the basis for a part of the interpretation appears to contain no mention of the stated subject matter.

[22] A. Bjursta, B. Hallgren and S. Ställberg-Stenhagen, unpublished investigations described by R. Ryhage and E. Stenhagen, Ref. 13, p. 415.

an ion multiplier and otherwise modified to give unit resolution at about 800. In the latter spectra quantitative variation from run to run was greater. M.ps were determined in open capillary tubes with a standardized thermometer. Optical rotations were determined in methanol solvent with a Bendix automatic polarimeter, type 143A, in 0·1-dm cells; limit of detection was 0·0002° of arc. Complete IR, NMR and mass spectra are recorded in the Ph.D. thesis of Donald W. Graham, Univ. of California, Berkeley, 1964.

Gas phase chromatography. Initial separations, involving large amounts, utilized an F. and M. instrument, Model 500, equipped with a stainless steel column of 1·9-cm i.d. which was straight except for a single U-turn. The 0·95-cm i.d. columns used for smaller amounts were spiral tubes of aluminum, and the instrument was an Aerograph A-90-P. Recorders of 1-mv span were used. Operating conditions and size of injections are specified in connection with the specific chromatographies described.

p-*Phthalimidophenacyl ester of* 3,7,11-*trimethyldodecanoic acid.* For preparation of the derivative of the *isolated acid*, a 75-mg sample of Frac. 1-D (cf. Fig. 1, Tracing B) was saponified by heating under reflux with 5 ml 10% methanolic KOH. Work-up yielded 60 mg acid which was dissolved in 2 ml purified dimethylformamide, treated with 85·3 mg *p*-phthalimidophenacyl bromide[24] and 70 μl dicyclohexylethylamine, then heated at 100° for 15 min. Dilution of the cooled solution with 70 μl 2N HCl and 10 ml water yielded a solid precipitate which was collected and dried, wt. 112 mg (90%), m.p. 92–99°. This derivative could not be crystallized satisfactorily from hexane, benzene, ether or carbon tetrachloride, or mixtures of these solvents, but acetone and water mixture proved satisfactory. Before crystallization of the derivative from the natural product, however, it was an advantage to first chromatograph it on 3·3 g Woelm alumina (neutral, activity 3). After elution with 10 ml benzene–hexane (1:1), which gave no eluted product, 20 ml benzene eluted 101 mg of product which was crystallized from acetone–water to yield 87 mg fine needles, m.p. 96–100°. Two additional crystallizations yielded 74 mg pure derivative, m.p. 99–101°. Concentration of the mother liquors yielded an additional 23 mg, m.p. 97–100°.

The derivative was prepared, by the same procedure, from 100 mg of the *synthetic acid* (cf. Table 1, footnote *c*) except that chromatography on alumina was omitted. The yield of crude product was 185 mg (92%), and two crystallizations from acetone-water yielded 81 mg fine needles, m.p. 99–101°, no depression on mixing with the derivative from the natural product. (Found: C, 73·9; H, 7·75. $C_{31}H_{39}O_5N$ requires: C, 73·6; H, 7·8%).

Methyl 3,7,11-*trimethyldodecanoate* was prepared form the synthetic acid by treatment with excess diazomethane. In gas chromatography, the retention times of the synthetic and natural esters were identical on both silicone and NPGS, and mixtures of the two gave a single symmetrical peak (operating conditions: 6 m × 0·95 cm columns, with He flow rate 150 ml/min; retention time 26·5 min with 10% GE-SF-96 at 175°, 51·5 min with 10% NPGS at 178°).

Thiourea adduction. Components of the methyl naphthenates were not adducted by thiourea in methanol unless an inducer, as recommended by Schlenk[25] was included. In a typical procedure, 13·3 g of methyl naphthenates (b.p. 135–147°/10 mm) were added to 125 ml methanol saturated with 20 g thiourea, then 10 ml 2,2,4-trimethylpentane (technical isooctane) was added and mixed well. The mixture was allowed to stand undisturbed for 12 hr at room temp (23°). There separated long needle-shaped crystals of the adduct which were collected, washed carefully with two 20-ml portions isooctane, pressed on the funnel and dried. The dried adduct was ground in a mortar then decomposed with 250 ml warm water. The liberated esters were extracted with three 75-ml portions ether, and the extracts were washed and dried. Removal of solvent left 1·2 g (9% recovery) of adducted esters. When the total filtrate from the thiourea adduct was treated with an additional 20 g thiourea and processed as described above, an additional 6% of adducted esters was obtained.

When the total esters obtained in the first adduction were again subjected to thiourea adduction, the yield of adducted esters was 14–23%, thus the over-all recovery of adducted esters was 2·1–3·5%. Gas chromatography of this twice-adducted material is shown in Fig. 2, Tracing A.

Isolation of methyl 2,6,10-*trimethylhendecanoate.* Since the yield in thiourea adduction of the distilled fraction, b.p. 135–147°/10 mm, was so low, it was necessary to find an additional source of the C_{14} isoprenoid ester, other than the chromatography shown in Fig. 2, Tracing B, in order to secure sufficient material for the NMR spectrum. It proved possible to isolate this ester without

[25] W. Schlenk, *Liebigs Ann.* **573**, 142 (1951).

thiourea adduction by removal of methyl dodecanoate by urea adduction. When the distilled fraction was gas chromatographed first on NPGS, then on silicone, the tracing differed from Tracing B, Fig. 2, only in that the first shoulder, at 11 min, was as large as the main peak in Tracing B and seriously overlapped it. When the material collected as in Cut 1, Tracing A, except from unadducted ester, was first treated with urea in isooctane containing methanol, and the adduct was removed, the unadducted ester yielded a gas chromatography tracing almost identical with Tracing B, Fig. 2. Ester collected at the main band was of essentially the same purity as that purified *via* thiourea adduction, as judged by the intensity of the ion peak at m/e 143 in the mass spectrum.

The isolated ester had the same retention time in gas chromatography as the synthetic ester described below, and a mixture of the two gave a single symmetrical peak (operating conditions: 1·5 m × 0·6 cm column packed with 20% GE-SF-96, temp 176°, He flow rate 71 ml/min, retention time 17·6 min; 6 m × 0·95 cm column packed with 10% NPGS, temp 193°, He flow rate 150 ml/min, retention time 15·6 min).

Farnesyl acetate. A mixture of 15 g farnesol (Givaudin), 20 ml acetic anhydride and 14 ml dry benzene was heated at 95° for 4 hr, then cooled and poured into 200 ml ice-water. Extraction of the product with ether and work-up yielded 14·0 g (79%) farnesyl acetate, b.p. 163–165°/10 mm; IR 5·76, 6·00 and 8·12 μ (lit.[24] b.p. 167–169°/10 mm).

Hexahydrofarnesyl acetate. A solution of 14·0 g farnesyl acetate in 130 ml methanol was hydrogenated at 1–2 atm. press. in presence of 0·2 g PtO_2 catalyst. When the theoretical amount of hydrogen had been consumed (about 2 hr), the catalyst was removed by filtration, the methanol solution was diluted with 400 ml water, and the product was extracted with two 75-ml portions ether. After the extract had been washed with water and bicarbonate solution, then dried, solvent was removed and the product was distilled through a 67-cm simple Podbielniak-type column at 10 mm pressure to yield three fractions: farnesane (2,6,10-trimethyldodecane), wt. 3·5 g, b.p. 118–120°, n_D^{25} 1·4316 (lit.,[24] b.p. 119·5–120°/10 mm, n_D^{25} 1·4303); intermediate frac., wt. 1·3 g; hexahydrofarnesyl acetate, wt. 5·4 g (38%), b.p. 158–162°, n_D^{25} 1·4405, IR 5·75, 8·07 μ. Gas chromatography of this mixture on a 6 m × 0·95 cm column packed with 10% silicone (temp 170°, He flow rate 175 ml/min) gave retention times of 7 min for farnesane and 26·2 min for hexahydrofarnesyl acetate.

3,7,11-*Trimethyl*-1-*dodecene.* A 5·1 g sample of hexahydrofarnesyl acetate was pyrolyzed at 510° essentially according to Bailey and Golden,[25] in a 1·2 × 22 cm Pyrex tube filled to a depth of 18 cm with 3-mm Pyrex helices. Dry nitrogen was swept through the tube at a rate of ca. 100 ml/min as the acetate was allowed to drop onto the heated helices at the rate of ca. 0·8 g/min. The pyrolysate was rinsed from the receiving trap with 50 ml ether, and the ether solution was washed with bicarbonate solution and water, then dried. Distillation through the 67-cm Podbielniak column at 12 mm pressure yielded 1·6 g (40%) of the desired alkene, b.p. 115–117°; n_D^{25} 1·4375; IR 3·28, 6·09, 10·02, 10·94 μ (lit.,[25a] b.p. 117–120°/12 mm, n_D^{12} 1·4398). A residue of 0·6 g was largely recovered acetate. A sample of distillate was analyzed (Found: C, 85·7; H, 14·2. Calc. for $C_{15}H_{30}$: C, 85·6; H, 14·4%.

Methyl 2,6,10-*trimethylhendecanoate.* Ozonolysis of the C_{15} alkene proved even less satisfactory than permanganate oxidation of the C_{20} alkene, although the elegant method of Knowles and Thompson was used to decompose the ozonide.[26] A 1-g sample (4·8 mmoles) of the C_{15} alkene in solution in 12 ml methanol and 12 ml methylene chloride was cooled in a dry ice-acetone bath as a stream containing 0·176 mmole ozone/min was passed in for 30 min (5·28 mmoles total ozone). To the cold solution was added 1 ml triethyl phosphite, then the solution was allowed to warm to room temp. After the solution had stood overnight solvent was removed at red. press., and a solution of the residue in 50 ml ether was washed with two 25-ml portions water, then dried. The residue obtained by removal of ether showed weak absorption in the carbonyl region (5·77 μ), intense absorption in the hydroxyl region (3·06 μ). This residue was dissolved in 25 ml acetone and stirred at room temp with 0·49 g permanganate for 3 hr. The resultant reaction mixture was diluted with water and treated with sufficient $NaHSO_3$ and H_2SO_4 to destroy the MnO_2, then the acidic solution was extracted with ether. After this solution had been extracted with three 20-ml portions 10% Na_2CO_3 solution, then washed and dried, removal of solvent left 0·75 g of neutral material which contained 5 major components (gas chromatography on 10% silicone, 162°, 6 m × 0·95 cm column).

[24] F. G. Fischer and K. Löwenberg, *Liebigs Ann.* **464**, 69 (1928).
[25] W. J. Bailey and H. R. Golden, *J. Amer. Chem. Soc.* **75**, 4780 (1952).
[25a] J. von Braun and E. Anton, *Ber. Dtsch. Chem. Ges.* **72**, 1490 (1929).
[26] W. S. Knowles and Q. E. Thompson, *J. Org. Chem.* **25**, 1031 (1960).

The combined carbonate extracts described above were acidified to Congo Red with 10% H_2SO_4, and the organic acid was extracted with three 15-ml portions ether. Removal of solvent from the washed and dried extract afforded 127 mg (13%) crude 2,6,10-trimethylhendecanoic acid. Esterification with methanol–H_2SO_4 yielded 114 mg crude ester whose gas chromatography showed it to be contaminated with ca. 10% lower molecular weight degradation products. Separation on a 6 m × 0·95 cm column packed with 10% silicone (temp 165°, He flow rate 150 ml/min) yielded 76 mg pure ester, retention time 16·4 min (Found: C, 74·3; H, 12·3%; M.W. 242, mass spec. $C_{15}H_{30}O_2$: requires: C, 74·3; H, 12·5%; M.W. 242).

Dihydrophytol. A solution of 50 g phytol (Matheson, "triple-distilled") in 130 ml dry methanol was hydrogenated, in presence of 0·6 g PtO_2 catalyst, at an initial pressure of 2·5 atm. After the calculated amount of hydrogen had been consumed (1·5 hr) the catalyst was removed by filtration and the solvent was distilled at red. press. Gas chromatography of the crude product on a 6 m × 0·95 cm column packed with 10% GE-SF-96 (temp 210°, He flow rate 150 ml/min) showed presence of about 6% phytane (ret. time 11·4 min) in addition to dihydrophytol (ret. time 26·0 min). Fractional distillation through the 67-cm Podbielniak column yielded a fore-run of 3·1 g, b.p. 166–169°/9·5 mm, and 42·1 g (86%) of dihydrophytol, b.p.199–200°/10 mm; n_D^{20} 1·4547; IR 3·05, 9·44; $[\alpha]_D^{23}$ ··· ··0·20 ·:· 0·02° (lit.,[27,28] n_D^{20} 1·4538; 1·4545).

Phytene. A mixture of 20 g dihydrophytol, 100 ml acetic anhydride and 6·0 g sodium acetate was heated at 100° for 3 hr. The cooled reaction mixture was poured on 400 g crushed ice, and the product was worked up by way of ether extraction. Distillation yielded 20·3 g (89%) of dihydrophytyl acetate, b.p. 169–173°/1·5 mm; IR 5·75, 8·10 μ.

A 10·5 g sample of acetate was cracked by the method described for the hexahydrofarnesyl acetate. Fractional distillation yielded 1·3 g fore-run, b.p. 103–160°/7 mm (g.c. indicated a complex mixture of lower mol. wt. comps), and 5·4 g (63%) phytene, b.p. 160–164°/7 mm; n_D^{25} 1·4445; IR 3·27, 6·09, 10·03, 10·96 μ (lit.,[29] n_D^{25} 1·4430). The residue of 1·1 g was about 75%, by g.c., unreacted acetate.

2,6,10,14-*Tetramethylpentadecanoic acid.* In a procedure similar to that described by Kaufmann and Stamm,[30] a stirred solution of 1·5 g phytene in 50 ml acetone (distilled from permanganate) was treated portionwise during 5 hr with 2·54 g powdered $KMnO_4$. After the mixture had been stirred for an additional 12 hr, gas chromatography indicated presence of large amounts of phytene; therefore, an additional 2·54 g of permanganate was added as before and stirring was continued another 12 hr, by which time all the phytene had been consumed (g.c.). MnO_2 was removed from the acidified solution by filtration, and the filter cake was washed well with acetone. The residue from removal of solvent from the total filtrate was dissolved in ether, washed with water, and passed through a Kies extraction tube[31] charged with a solution of 0·8 g KOH in water–methanol (80:20). Acid recovered from the alkaline extract amounted to 0·52 g (33%), n_D^{25} 1·4495 (lit.,[29] 1·4489). A sample of pure amide could not be obtained in a preparation directly from this crude acid. It was first necessary to remove about 5% of low M.W. impurities by g.c. of the ester, as below.

Methyl 2,6,10,14-*tetramethylpentadecanoate.* A 206-mg sample of crude acid was esterified by heating under reflux with methanol containing 10% H_2SO_4. Gas chromatography on a 6 m × 0·95 cm column containing 10% GE-SF-96 silicone (temp 210°, He flow rate 177 ml/min) yielded 142 mg pure ester, ret. time 16·2 min. The isolated ester of the C_{19} isoprenoid acid gave an identical retention time on the silicone column as well as on a 6 m × 0·95 cm column packed with 10% NPGS, where the ret. time was 36·3 min at 200°, He flow rate 150 ml/min. The isolated and synthetic esters also had the same mass spectra, with a single molecular ion at 312.

2,6,10,14-*Tetramethylpentadecanamide.* A 76-mg sample of the pure ester was saponified with methanolic KOH, and the resultant acid was added to 1.5 ml dry benzene and 0·75 ml purified thionyl chloride. The resultant reaction mixture was allowed to stand at room temp for 1·5 hr, then heated at 60° for 1 hr. After solvent and excess reagent had been removed by heating to 50° at red. press. the residue was dissolved in 4·5 ml dioxane (water and peroxides removed from dioxane by

[27] R. Kuhn and H. Sugmoine, *Helv. Chim. Acta* **12**, 916 (1929).
[28] V. Kvita and J. Weichet, *Coll. Czech. Chem. Comm.* **25**, 254 (1960).
[29] L. I. Smith and G. A. Boyack, *J. Amer. Chem. Soc.* **70**, 2690 (1948).
[30] H. P. Kaufmann and W. Stamm, *Chem. Ber.* **91**, 2121 (1958).
[31] M. W. Kies and P. L. Davis, *J. Biol. Chem.* **189**, 637 (1951).

percolation through a column of basic Woelm alumina). The acid chloride solution was added dropwise from a syringe, with swirling, to 20 ml ice-cold conc NH_4OH. The precipitate of crude amide was collected, washed and dried; wt. 22 mg, m.p. 63–66°. One crystallization from hexane at −10° yielded 9·2 mg pure amide, m.p. 64–66° (Found: N, 4·7. $C_{19}H_{39}ON$ requires: N, 4·7%).

A 51·6-mg sample of the isolated ester of the C_{19} isoprenoid acid was saponified and converted to the amide as described for the synthetic acid. The crude product weighed 17 mg, and after three crystallizations from hexane at −10° there was obtained 1 mg pure amide, m.p. 63–65°, no depression on mixing with the synthetic sample. The mother liquors yielded an additional 6 mg of amide, m.p. 62–63°.

3,7,11,15-*Tetramethylhexadecanoic acid.* In a procedure similar to that described by Karrer *et al.*,[32] a solution of 4·67 g of CrO_3 and 4·67 g $KHSO_4$ in 80% acetic acid (distilled from permanganate) was added over a period of 45 min to a stirred solution of 10 g dihydrophytol in 70 ml acetic acid. The temp rose to 40° during the addition, as a green precipitate formed. After the mixture had been stirred for an additional 3 hr at room temp it was diluted with 300 ml water and extracted with three 50-ml portions pet. ether. The residue from this extract, after washing, drying and evaporation of solvent, weighed 5 g. Its IR spectrum indicated a complex mixture of esters (5·76 μ), ketones (5·85 μ) and acids (5·85 μ, broad adsorption at 3·5 μ). Distillation at 4 mm press. yielded: 0·2 g, b.p. 171–183°; 0·3 g, b.p. 183–200°; 0·3 g, b.p. 200–205°; 4·0 g residue. Gas chromatography of the distilled fractions indicated a complex mixture (9 peaks in frac. 1, 6 in frac. 2, 3 in frac. 3) of lower M.W. oxidation products.

The residue from distillation, which consisted largely of ester (IR) was saponified with KOH in methanol. Work-up for separation of acid required Kies extraction[31] to avoid stable emulsions. The yield of crude acid was 1·9 g (18%), n_D^{20} 1·4543 (lit[28] n_D^{20} 1·4560).

Methyl 3,7,11,15-*tetramethylhexadecanoate* was prepared by esterification of 503 mg of the acid with methanol containing 10% by wt. of H_2SO_4. Work-up yielded 482 mg ester, n_D^{20} 1·4464, $[\alpha]_D^{28} = -0{\cdot}17 \pm 0{\cdot}02°$, single molecular ion in the mass spec at 326. This synthetic ester and the isolated ester of the C_{20} isoprenoid acid had the same retention times in gas chromatography, gave a single symmetrical peak on mixing (ret. time 22·2 min on 6 m × 0·95 cm column packed with 10% GE-SF-96, temp 212°, He flow rate 150 ml/min; ret. time 46·9 min on 6 m × 0·95 cm column packed with 10% NPGS, temp 217°, He flow rate 200 ml/min).

3,7,11,15-*Tetramethylhexadecanamide.* Preparation of the amide from 195 mg of acid, by the procedure described for the C_{20} acid, gave 43 mg of crude amide of m.p. 50–53°. Two crystallizations from aq. acetone gave 21 mg pure amide, m.p. 51–53° (lit[33] m.p. 53–53·5°) (Found: N, 4.3%. Calc. for $C_{20}H_{41}ON$: N, 4·3%). Attempted crystallization from hexane, ether or methanol failed.

A 63-mg sample of the isolated ester of the C_{20} isoprenoid acid was saponified and converted to the amide in the usual way to give 24 mg of crude amide. Three crystallizations from aq. acetone gave 8 mg pure amide, m.p. 51–53°, no depression on mixing with the sample of synthetic amide. Both samples of amide (cf. Table 2) showed a single molecular ion in the mass spec at 311.

p-*Phthalimidophenacyl ester of* 3,7,11,15-*tetramethylhexadecanoic acid.* Preparation of this derivative, as described for the C_{16} acid, from 150 mg acid, followed by three crystallizations from aq. acetone, yielded 52 mg, m.p. 99–101° (Found: C, 75·0; H, 8·6; N, 2·7%. $C_{36}H_{49}O_5N$ requires: C, 75·1; H, 8·6, N, 2·4%).

Preparation of the *p*-phthalimidophenacyl ester of the isolated C_{20} isoprenoid acid utilized the acid from saponification of 88 mg of the isolated ester. After three crystallizations from aq. acetone, there was obtained 27 mg, m.p. 99–101°, no depression on mixing with the synthetic sample. It is of interest that there was also no depression in m.p. on mixing with this derivative of the C_{16} isoprenoid acid.

[32] P. Karrer, A. Epprecht and H. König, *Helv. Chim. Acta* **23**, 272 (1940).
[33] R. Willstätter, E. W. Mayer and E. Hüni, *Liebigs Ann.* **378**, 105 (1910).