ditions of hydrolysis. The procedure has been used routinely to check the amino acid content of peptides prepared in a program devoted to synthesis of hormone and enzyme-active-site analogs.

Quantitative Aspects. The relative peak areas for most of the amino acid derivatives remained remarkably constant during six repetitions of the procedure with a standard mixture of amino acids. All but two of the 20 amino acids analyzed gave reproducible peak area ratios (based on trifluoroacetylalanine methyl ester = 1.00) within ± 10% at a 95% confidence limit; of these, 10 were within ± 5% (Table V). Poor reproducibility with cystine and histidine was probably due to decomposition of the sensitive side chain functional groups (sulfhydryl and imidazolyl, respectively).

Through utilization of these peak area ratios, determination of amino acid ratios in hydrolysates of peptides of moderate complexity is possible. By including as an internal standard a known amount of an amino acid not present in the sample under investigation, a true quantitative analysis could be carried out.

Table V. Peak Area Ratios for Trifluoroacetylated Amino Acid Methyl Esters[a]

Amino acid	Peak area ratio[b,c]	95% Confidence limits	Amino acid	Peak area ratio[b,c]	95% Confidence limits
Alanine	1.00	...	Arginine	0.55	± 8.1%
Valine	1.07	± 2.3%	Histidine	0.48	±15.7%
Isoleucine	1.14	± 3.6%	Cystine	0.46	± 9.7%
Glycine	0.96	± 2.0%			
Threonine	0.63	± 8.1%			
Leucine	2.12	± 2.9%			
Proline	1.85	± 3.5%			
Serine	0.49	± 6.0%			
Aspartic acid	1.57	± 3.8%			
Cysteine	0.26	±30.0%			
Hydroxyproline	1.32	± 4.6%			
Methionine	3.69	± 3.3%			
Glutamic acid	1.45	± 8.4%			
Phenylalanine	7.39	± 2.3%			
Tyrosine	3.38	± 4.4%			
Lysine	3.70	± 8.7%			
Tryptophan	3.74	± 6.6%			

[a] Characteristic for Jarrell-Ash Argon Diode Detector, Model 26-752, Serial no. 126262, with 20 mc. of Sr^{90}. Chromatography conditions were described in Figure 1, except 2 μl. of a 0.01*M* solution containing derivatives obtained from a 0.01*M* amino acid standard solution was used.
[b] Relative to trifluoroacetylalanine methyl ester.
[c] Peak areas were determined automatically with a Texas Instruments Servo/Riter integrating recorder.

LITERATURE CITED

(1) Bellamy, L. J., "The Infrared Spectra of Complex Molecules," 2nd ed., Wiley, New York, 1958.
(2) Beyer, E., "Gas Chromatography," D. H. Desty, ed., p. 333, Academic Press, New York, 1958.
(3) Black, R. J., Durrum, E. L., Zweig, G., "Paper Chromatography and Paper Electrophoresis," 2nd ed., Academic Press, New York, 1958.
(4) Gross, D., Grodsky, G., *J. Am. Chem. Soc.* **77,** 1678 (1955).
(5) Johnson, D. E., Scott, S. J., Meister, A., ANAL. CHEM. **33,** 669 (1961).
(6) Kyrides, L. P., *J. Am. Chem. Soc.* **66,** 1006 (1944).
(7) Levy, A. L., Chung, D., ANAL. CHEM. **25,** 396 (1953).
(8) Peiz, K. A., Morris, L., *Anal. Biochem.* **1,** 187 (1960).
(9) Saraff, H. A., Karmen, A., *Ibid.*, **1,** 344 (1960).
(10) Schwartz, D. P., Pallansch, M. J., ANAL. CHEM. **30,** 219 (1958).
(11) Spackman, D. H., Stein, W. H., Moore, S., *Ibid.*, **30,** 1190 (1958).
(12) Voss, W., Blanke, E., *Ann.* **485,** 258 (1931).
(13) Wagner, J., Winkler, G., *Z. Anal. Chem.* **183,** 1 (1961).
(14) Weygand, F., Kolb, B., Prox, A., Tilak, M. A., Tomida, I., *Z. Physiol. Chem.* **322,** 38 (1960).
(15) Zomzely, C., Marco, G., Emery, E., ANAL. CHEM. **34,** 1414 (1962).

RECEIVED for review June 12, 1963. Resubmitted March 9, 1964. Accepted March 9, 1964.

Composition of Straight Chain Alkylbenzenes by Gas Chromatography

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▶ Apiezon-L, SE-30, and DC-550 were evaluated as liquid coatings on 150-foot, 0.01-inch capillary columns. The best separation of components was achieved with a DC-550 liquid substrate and temperature increased uniformly at 1.5° per minute between 120° and 170° C. Linear alkylbenzenes with side chain ranging from C_9 to C_{14} were completely separated in 65 minutes. The isomers arising from the position of phenyl attachment were resolved, except for 7- and 6-phenyl substituted alkanes. A criterion for judging column performance is included.

THE MOST widely used detergent raw material has been alkylbenzene prepared by alkylation of benzene with propylene tetramer. The alkylbenzenes produced are primarily dodecylbenzene isomers but also contain varying amounts of alkylbenzene with chain lengths ranging from C_{10} to C_{15}. Up to 80,000 isomers are possible in the final mixture (*3*), and the complete characterization of a typical alkylbenzene detergent alkylate has not been accomplished. Gas chromatography can be applied, but the samples are so complex that separation and identification of components is not possible. The best that has been achieved is a general profile chromatogram indicating the molecular weight range. The chromatogram of a typical tetrapropylene-derived detergent alkylate is shown in Figure 1. Since tetrapropylbenzene sulfonate is relatively resistant to biodegradation, manufacturers of detergent alkylate have sought a detergent alkylate that is readily biodegradable.

Alkylbenzene produced by reaction of 1-olefins with benzene is now available. This material is primarily straight side-chain isomers having chain lengths of C_{10} to C_{14}. Length of side chain and position of attachment of the phenyl group mean 26 compounds are possible. Under these more favorable circumstances efforts to establish a gas chromatographic method of analysis for straight chain alkylbenzene raw materials were worthwhile. Both chain length and position of phenyl substitution must be specified because they affect detergency characteristics.

Packed column gas chromatographic methods for analyzing detergent alkylates (*1, 2, 6, 8, 10*) resulted in partial or no resolution of the higher chain length isomers when applied to typical straight chain alkylbenzenes. Analysis times were also excessive for a convenient routine analysis schedule applicable to a large number of samples. Capillary columns have also been used for the

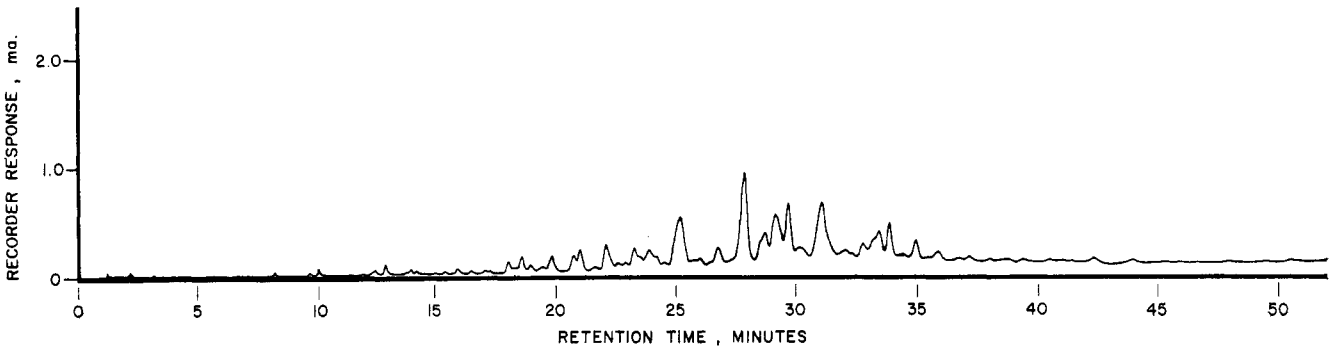

Figure 1. Chromatogram of tetrapropylene derived phenyl dodecane. Stationary phase DC-550

analysis of alkylbenzenes (*4*). Swisher, Kaelble, and Liu (*11*) studied the alkylation reaction and accompanying isomerization using pure phenyl dodecane. Separations were made with a 200-foot, 0.01-inch capillary column coated with Apiezon-L. Silver *et al.* (*9*) applied capillary column techniques to obtain average molecular weight information on commercial tetrapropylene derived detergent alkylates. Since none of these methods provided the separation needed to characterize commercial straight chain alkylbenzenes, the present study was undertaken.

A comprehensive method of analysis is described to provide isomer content over a broad chain-length range. Three columns were compared for the separation of straight chain alkylbenzenes. A definite improvement in isomer separation, particularly the 2-phenyl isomer, was achieved by the use of DC-550 (polar silicone oil) as the liquid phase in preference to Apiezon-L or SE-30. The method is suitable for routine quality control and can be utilized in the manufacturing plants.

EXPERIMENTAL

Apparatus. The Perkin-Elmer Model 226 programmed-temperature gas chromatograph was used in this work. On this instrument the proper column loading is obtained by a fixed orifice stream splitter controlled by a valve that vents a portion of the injected sample to the atmosphere. Hypodermic capillary columns are coiled in a "pancake" configuration and housed between low mass aluminum disks. Column heating is provided by a direct thermal contact of the column unit to a matching low-mass aluminum heater block. The column heating and cooling cycle is completely automatic. The detector is a hydrogen flame detector with a negative biased detector jet to remove positive ions and to eliminate ion recombination. The output from the detector may be used with a galvanometric or a potentiometric recorder. The helium carrier gas is cleaned by passing it through a cartridge of activated molecular sieves. Matheson prepurified air and National Cylinder Gas Corp. hydrogen are used to supply the detector burner.

Columns. Stainless steel 150-foot, 0.01-inch capillary columns were obtained precoated from Perkin-Elmer Corp. They were ready for use after preconditioning for 12 hours under 40 p.s.i.g. helium. Three liquid phases were evaluated for the separation of alkylbenzenes. Apiezon-L (low vapor pressure hydrocarbon grease) and other high molecular weight hydrocarbon liquid phases have been used extensively for the separation of alkylbenzenes (*1*, *2*, *6*, *9–11*). Alumina-washed Apiezon-L, SE-30 (methyl silicone rubber) and DC-550 (polar methylphenyl silicone oil) were compared for the separation of alkylbenzene isomers.

Detector Operation. Air and hydrogen pressures were set to provide a maximum detector signal when helium containing 0.95% isobutane was passed through the detector carrier gas inlet. The particular air and hydrogen pressures used depended on the individual detector burner jet assembly. Air pressure was between 40 to 50 p.s.i.g., and the hydrogen pressure, between 10 and 20 p.s.i.g.

Signal Recording and Automatic Integration. A 5-ma. galvanometric recorder was used and because of the large number of peaks encountered, areas were calculated by an automatic peak integration system. The CRS-20 data processing equipment (Infotronics Corp., 1401 S. Post Oak Road, Houston 27, Tex.) records and prints individual peak areas and retention times. A detailed explanation of the CRS-20 system is beyond the scope of this discussion, but the equipment includes the following units:

An on-line hi-fidelity tape recording deck with a voltage-to-frequency tone converter accepts the voltage output of the detector that is normally directed to a potentiometric recorder. The voltage-to-frequency converter generates an audio signal which is recorded on magnetic tape. Linear recording ranges from 2 to 50 mv. can be selected on the tape recorder. A tape transport speed of $1^7/_8$ inches per second is used.

An off-line playback tape recorder accepts the tape recordings for playback of the chromatogram. The tapes are played back at $7^1/_2$ inches per second, providing a four-fold time compression of the chromatogram. Upon playback the audio signal is reconverted to voltage to provide signals to the integrating circuit.

The integrator and buffer memory unit provide a digital readout of peak

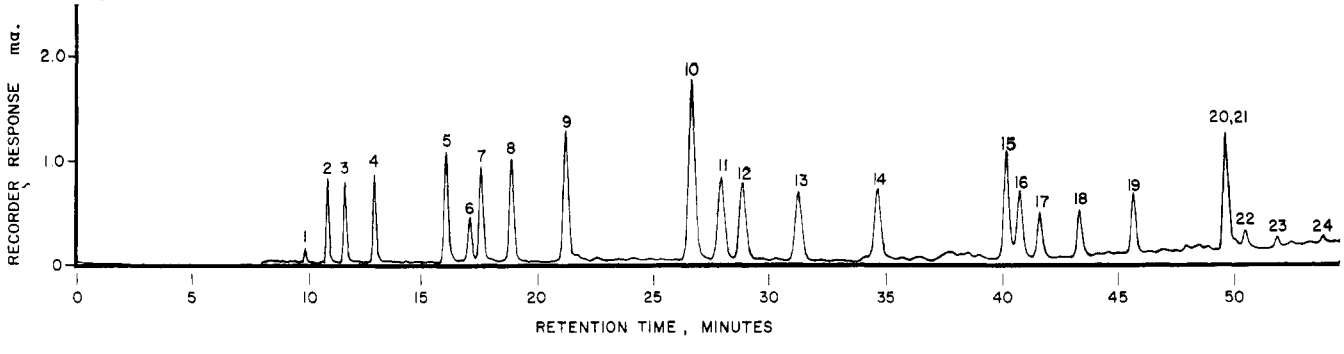

Figure 2. Chromatogram of a straight chain alkylbenzene. Stationary phase Apiezon-L (see Table I for conditions). Peak numbers refer to Table II

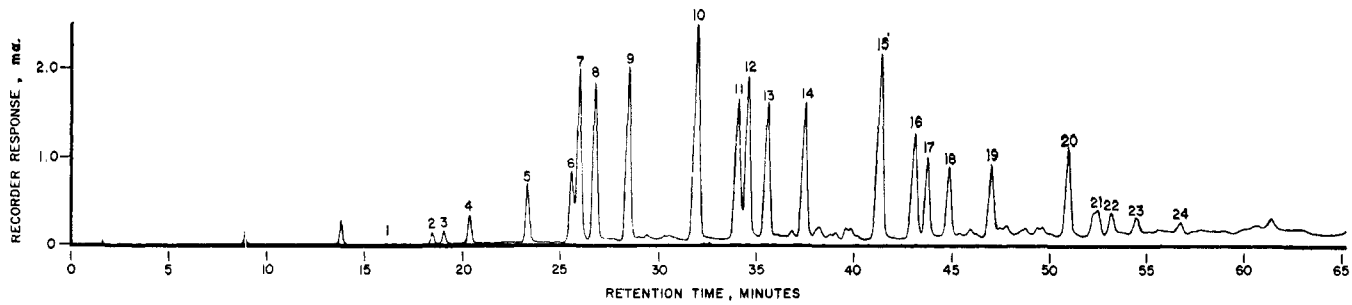

Figure 3. Chromatogram of a straight chain alkylbenzene. Stationary phase DC-550 (see Table I for conditions). Peak numbers refer to Table II

area, in counts per second, retention time in seconds and the attenuation factor setting used during the recording of the signal. Peak detection based on minimum slope change may be selected by setting a dial on the front panel of the CRS-20 unit. An automatic baseline offset correction unit is incorporated in the integration circuitry to compensate for baseline drift during the analysis.

A description of the Infotronics system has been given (*5*), and a manuscript is being prepared for publication.

Instrument Conditions. Operating conditions were established for the separation of isomers using Apiezon-L, SE-30, and DC-550 as liquid column coatings. In establishing the conditions, goals were: All isomeric species should be resolved for all chain lengths from phenyl decane through phenyl tetradecane. Analysis times should be less than 80 minutes.

To satisfy the time limitations, 150-foot columns were used. Good separations were obtained using a 300-foot column but analysis times were over 2 hours. The instrument conditions established for each column are listed in Table I.

Peak Identification. The boiling points and carbon number of the straight chain alkylbenzenes are linearly related to the logarithm of retention time (*10*). Peak identifications are routinely made by reference to a chromatogram of a standard alkylbenzene sample. During development of the analytical method, identifications were made by comparing relative retention times with samples of known composition. Pure isomers of phenyl dodecane were used to verify the order of elution of the phenyl isomers. The 1-phenyl isomer has not been found in any of the samples analyzed.

RESULTS AND DISCUSSION

The separations achieved on Apiezon-L are illustrated in Figure 2. To obtain the maximum resolution and separation over a wide chain length distribution, a combination of isothermal and linear temperature programming was used. The starting column temperature and the length of the initial delay had the largest effect upon the resolution of the various isomers. A compromise was made between analysis time and resolution. Under the conditions finally chosen a better separation of the 2-phenyldodecane from the 7- and 6- phenyl tridecanes was sacrificed to provide a run time of approximately 70 minutes. Complete resolution of the 2-phenyl isomers was obtained except for the 2-phenyl tridecane and higher 2-phenyl isomers.

The separations achieved using DC-550 are illustrated in Figure 3. By using this column, complete separation of the 2-phenyl isomers for all chain lengths was achieved. The resolution of the internal isomers (7-, 6-, and 5-phenyl isomers) was less than that achieved with Apiezon-L, but there was no overlapping of phenyl isomers between chain lengths. The analysis time was 65 minutes for chain lengths through C_{14}.

Table I. Analytical Conditions for Analysis of Straight Chain Alkylbenzenes

Conditions	Liquid Phase: Apiezon-L	SE-30	DC-550
Inlet temp., ° C.	300	300	300
Initial col. temp., ° C.	150	110	120
Initial hold period, min.	30	0	0
Program rate, ° C./min.	2	1.5	1.5
Final temp., ° C.	220	250	170
Final hold period, min.	30	0	30
Carrier gas, p.s.i.g.	60	60	60
Split ratio	300:1	300:1	300:1
Sample size, μl.	0.6	2	1
Attenuation	10	100	100

Illustrated in Figure 4 are the separations achieved on SE-30. Separation of the internal phenyl isomers was lost, but the separation of the 2-phenyl isomers was still accomplished. SE-30 provides less separation than DC-550.

Log relative retention time plots for the three liquid substrates show a linear relation with carbon number. DC-550 is the best liquid phase for the separation of straight chain alkylbenzenes. Although DC-550 is a polar substrate, no evidence of a selective separation of any of the phenyl isomers

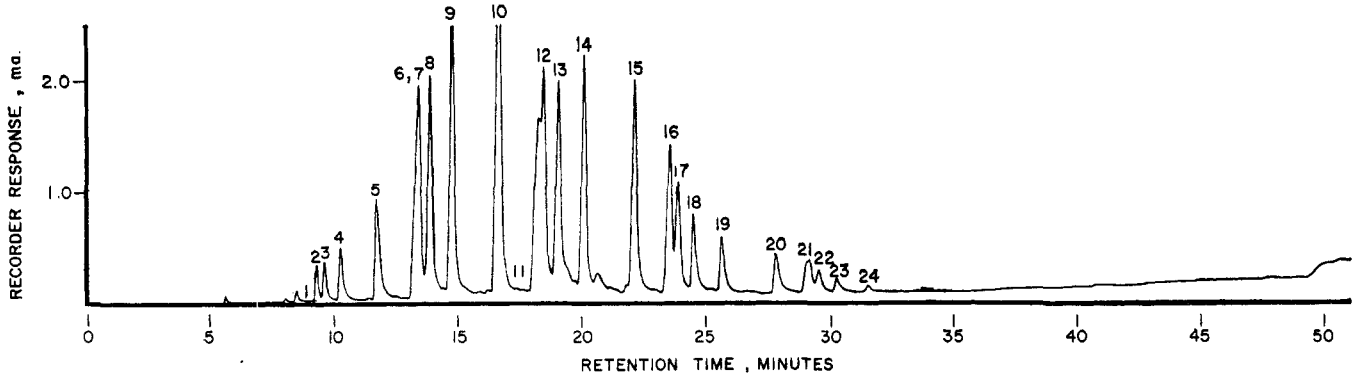

Figure 4. Chromatogram of a straight chain alkylbenzene. Stationary phase SE-30 (see Table I for conditions). Peak numbers refer to Table II

are evident when using DC-550. Apparently, DC-550 falls into an intermediate position between Apiezon-L and SE-30 as far as the relative separation obtained. Apiezon-L actually provides too much separation between isomers, and the 2-phenyl isomers overlap the 7-, 6-, and 5-phenyl isomers of the next higher chain length.

Table II. Composition of Straight Chain Phenyl Alkane Mixture (See Figure 3)

Results from 8 replicate analyses

Peak No.	Component	Mean weight, %	σ
1	2-Phenyl nonane	0.098	0.02
2	5-Phenyl decane	0.74	0.10
3	4-Phenyl decane	0.86	0.06
4	3-Phenyl decane	1.40	0.06
5	2-Phenyl decane	3.28	0.17
6	6-Phenyl undecane	2.77	0.10
7	5-Phenyl undecane	5.08	0.22
8	4-Phenyl undecane	5.64	0.19
9	3-Phenyl undecane	7.62	0.26
10	2-Phenyl undecane	12.14	0.53
11	6-Phenyl dodecane	5.82	0.13
12	5-Phenyl dodecane	5.66	0.08
13	4-Phenyl dodecane	5.96	0.18
14	3-Phenyl dodecane	6.86	0.16
15	2-Phenyl dodecane	7.83	0.14
16	7-,6-Phenyl tridecane	4.75	0.15
17	5-Phenyl tridecane	2.92	0.12
18	4-Phenyl tridecane	2.68	0.14
19	3-Phenyl tridecane	2.12	0.17
20	2-Phenyl tridecane	2.00	0.11
21	7-, 6-Phenyl tetradecane	1.65	0.12
22	5-Phenyl tetradecane	0.88	0.13
23	4-Phenyl tetradecane	0.83	0.23
24	3-Phenyl tetradecane	0.49	0.17
	Branched isomers and other hydrocarbons	10.00	...
		100.00	

Analytical Results. Precision data for a typical alkylbenzene analysis, using DC-550, are given in Table II. These results were obtained using area normalization of the individual peak areas obtained from the CRS-20 digital integrator. The standard deviations represent the total variation inherent in the gas chromatograph and the CRS-20 integrator. The value for branched isomers and other hydrocarbons was obtained by difference. Some variation in analytical results for the various components was seen between columns with different liquid substrates. This was attributed to the difference in relative retention volumes of the straight chain material and the branched chain materials on different liquid substrates. Certainly the pattern of the branched chain isomers from chromatograms on different substrates is very much different, and differences in resolution of the branched isomers from the straight chain isomers would cause minor variations in the apparent straight chain isomer levels.

Isomer Distribution. Analysis of samples from various producers led to the conclusion that there are two types of isomer distribution. Presumably the differences arise in the manufacturing process. The two kinds of alkylbenzenes have been designated as "high-two" and "flat" isomer distributions, and compositions of both types are given in Table III. It has also been observed that the ratio of the 3-phenyl isomer to the 4-phenyl isomer averaged for all chain lengths is greater than unity for the "high-two" isomer distribution and less than unity for the "flat" distribution. Generally the level of 5-phenyl isomer is slightly higher than that for either the 4-phenyl or the 6-phenyl isomer. The isomer distribution does not vary significantly among chain lengths, except for the shortest and longest chain lengths. In general, the relative isomer ratios are different at the shorter and longer chain lengths from those of the central chain length.

Branched Chain Isomers. The sample size and chromatographic conditions necessary to obtain the proper resolution of straight chain isomers often reduces the size of the branched alkylbenzene peaks to the point where they are difficult to measure accurately. The presence of some branched chain material is evident because the sum of all the straight chain isomers is equivalent to only about 95% of the total peak areas of the chromatogram. In the samples analyzed, the branched chain content generally ranged between 5 to 10%.

Column Performance. The criterion for judging the performance of DC-550 columns is the resolution of the 6- and 5-phenyl undecanes. Varying degrees of separation have been observed with different columns of identical length and bore, and having the same liquid coating. Similar behavior of capillary columns has been reported by other workers (7). In our experience a change in starting temperature, program rate, and column inlet pressure may be required to attain the proper resolution when a new column is installed.

Table III. Weight Percentages of Straight Chain Alkylbenzene Isomers

	Phenyl Position				
	7-, 6-	5-	4-	3-	2-
		"High Two" Isomer Distribution			
Sample A					
C_{10}		0.46	0.45	0.80	1.47
C_{11}	1.51	3.40	3.46	4.70	7.07
C_{12}	5.05	5.57	4.97	6.45	11.78
Sample B					
C_{10}		5.85	4.60	6.22	10.55
C_{11}	2.22	4.25	3.90	5.16	9.29
C_{12}	3.29	3.44	3.39	4.34	8.97
		"Flat" Isomer Distribution			
Sample C					
C_{10}		0.07	0.08	0.12	0.46
C_{11}	1.48	2.62	2.37	1.97	2.13
C_{12}	6.11	5.87	4.27	3.94	3.25
Sample D					
C_{10}		0.1	0.1	0.2	0.4
C_{11}	2.4	4.9	3.8	3.4	3.0
C_{12}	6.6	6.4	4.5	3.9	3.4

LITERATURE CITED

(1) Blakeway, J. M., Thomas, D. B., *J. Chromatog.* **6**, 74 (1961).
(2) Chang, T. C. L., Karr, C., *Anal. Chim. Acta* **21**, 475 (1959).
(3) *Chem. Eng. News* **41**, No. 11, 103 (1963).
(4) Kaelble, E. F., *Soap Chem. Specialties* **39**, 56 (1963).
(5) Karohl, J. G., Seventh Conference on Analytical Chemistry in Nuclear Technology, Gatlinburg, Tenn., October 10, 1963.
(6) Olson, A. C., *Ind. Eng. Chem.* **52**, 833 (1960).
(7) Schwartz, R. D., Basseaux, D. J., Shoemake, G. R., ANAL. CHEM. **35**, 496 (1963).
(8) Setzkorn, E. A., Carel, A. B., *J. Am. Oil Chemists' Soc.* **40**, 57 (1963).
(9) Silver, A. H., Adam, W. H., Gardner, H. M. Keily, H. J., *Ibid.*, **38**, 674, (1961).
(10) Spencer, C. F., Johnson, J. F., *J. Chromatog.* **4**, 244 (1960).
(11) Swisher, R. D., Kaelble, E. F., Liu, S. K., *J. Org. Chem.* **26**, 4066 (1961).

RECEIVED for review January 30, 1964. Accepted April 6, 1964.