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INFLORESCENCE HYDROCARBONS OF SOME SPECIES OF *SOLANUM* L., AND THEIR POSSIBLE TAXONOMIC SIGNIFICANCE*

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Abstract—A study of the hydrocarbon content of inflorescences of twenty-two tuber-bearing *Solanum* species has yielded results applicable to the taxonomy of this group. The alkanes, which occur as a portion of the waxy coating on the surface of the plant parts, were purified and the relative amounts of the various components determined using gas–liquid chromatography. All species examined were found to contain both branched (C_{25}–C_{32}) and normal (C_{25}–C_{31}) components.

INTRODUCTION

THE tuber-bearing species of *Solanum* L., vernacularly known as potatoes, occur in nature in both North and South America. The 157 species recognized by Correll[1] in *Solanum*, section Tuberarium, subsection Hyperbasarthrum, form a polyploid series with the base number $x=12$. In addition to diploids, tetraploids, and hexaploids, triploids and at least one pentaploid have been identified. Taxonomists working with this complex have found it advisable to divide it into a number of series.[1,2]

The advantages of the alkane fraction of leaf waxes as a systematic criterion have been brought out by Eglinton and coworkers.[3–5] These include universal occurrence, species variation in composition, lack of seasonal variation, simplicity of sampling, and the availability of tools for their rapid analysis.

Reported here is an attempt to determine the applicability of hydrocarbon content of the inflorescence to the biosystematics of potatoes. It was hoped that a chemo-systematic approach might serve in elucidating relationships between the diploids and polyploids, and between the South American and Mexican species. It was hoped also that this approach might reflect on the validity of the series assignments.

Tubers and/or seeds of forty-two species of *Solanum* were obtained from the Inter-Regional Potato Introduction Station, Sturgeon Bay, Wisconsin, and grown in a greenhouse. Some of these foreign introductions were also grown in a field. Material from field-grown commercial varieties was also examined. Hydrocarbons extracted from the inflorescences of these plants showed species variation, but very little environmental variation, and so were chosen as a basis for this study.

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† N.S.F. Cooperative Fellow, 1964–65.

[1] D. S. CORRELL, *Contrib. Tex. Res. Found.* vol. 4 (1962).
[2] J. G. HAWKES, *Handbuch der Pflanzenzuchtung*, 2. Aufl., Bd. 3, p. 1 (1958).
[3] G. EGLINTON, A. G. GONZALES, R. J. HAMILTON and R. A. RAFAEL, *Phytochem.* 1, 89 (1962).
[4] G. EGLINTON, R. J. HAMILTON and M. MARTIN-SMITH, *Phytochem.* 1, 137 (1962).
[5] G. EGLINTON and R. J. HAMILTON, In *Chemical Plant Taxonomy* (Edited by T. SWAIN). Academic Press, New York (1963).

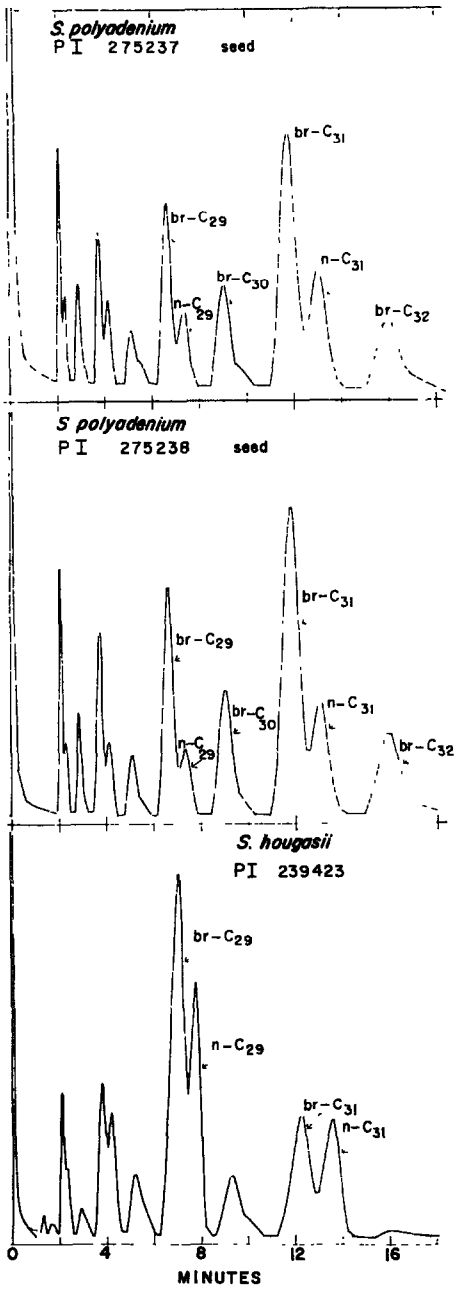

FIG. 1. REPRESENTATIVE CHROMATOGRAMS OF HYDROCARBONS OBTAINED FROM THE INFLORESCENCES OF TWO CLONES OF *S. polyadenium* AND ONE OF *S. hougasii*.

Run on an F and M Model 400 gas chromatograph fitted with a 1·25 m, 6 mm column containing 3·8% SE30 on Gas Chrom Q, 80–100 mesh. Column temperature, 230°; flow rate of the carrier (Helium), 60 ml/min.

RESULTS

Surface waxes were extracted by agitating the dried flower samples in chloroform for 1 hr. After evaporation of the chloroform, the hydrocarbons were separated from the more polar constituents of the mixture by column chromatography. The eluant containing the hydrocarbon fraction was concentrated for gas–liquid chromatographic analysis. Periodic checks utilizing thin-layer chromatography showed the presence in the samples of saturated hydrocarbons only.

The chromatograms produced showed two series of peaks. Comparison of retention times with known compounds indicated that one of these series represented straight-chain saturated hydrocarbons, the odd-numbered predominating. The second series represented branched-chain alkanes. These may be iso (2-methyl) or anteiso (3-methyl) homologs, or a mixture of both, as reported for tobacco by Mold *et al.*[6]

In the following discussion, a particular normal saturated hydrocarbon is referred to as n-C_n, or n-odd or n-even of a particular set; a branched compound is referred to as br-C_n.

Three representative chromatograms are shown in Fig. 1. Figure 1*a* depicts the hydrocarbon content of the flowers of *S. polyadenium*, P.I. 275237, grown from seed under glass. Figure 1*b* is of the same species, but a different clone (P.I. 275238). In contrast, the differences observed in the pattern of peaks obtained from *S. hougasii*, P.I. 239423, may be noted.

Due to the very small amounts of n-even components, the peaks seem to be arranged in four sets of three peaks each. The center peak in each set is an n-odd component; the peak immediately preceding it is a branched hydrocarbon of the same carbon number; that following is a branched component of the next higher carbon number. The area under each curve, which is proportionate to the amount of material, was determined from integrator pen tracings and calculated as a percentage of the total of the four sets. Bar graphs depicting these percentages are given in Fig. 2.

To check the reproducibility of this method, five extractions of one clone of *S. kurtzianum* were compared. Essentially similar results from these five runs indicated that the relative amounts of material were not affected by the length of time the samples had been stored (up to 2 years), or the stage of development of the flower when picked. Colchicine-induced polyploids gave results essentially similar to those obtained from the parent material.

DISCUSSION

The commercial varieties of *S. tuberosum*, in contrast to most of the wild species examined, had a large amount of material in the br-C_{25} peak, this sometimes being the constituent in greatest amount. With the exception of the n-C_{31} peak of Russet Burbank, no peak from a commercial variety exceeded 18 per cent of the total. Graph 1 (of Fig. 2) is representative of Russet Burbank, Graph 2 of the other commercial varieties examined.

Species in series Transaequatorialia, Acaulia, and Tuberosa showed as much interspecific as intraspecific variability, and will not be further discussed here.*

S. capsicibaccatum, of the South American series Circaeifolia, morphologically resembles *S. trifidum*, found in Mexico.[1] Its hydrocarbon pattern, however, shows close affinity to that found for *S. demissum* (Graph 3, Fig. 2).

* Examined—Series Acaulia: *S. acaule*; series Transaequatorialia: *S. brevicaule*, *S. canasense*, *S. canasense* var. *neohawskii*, *S. candolleanum*, *S. kurtzianum*, *S. lignicaule*, *S. simplicifolium*, *S. soukupii*, *S. sparsipilim*, *S. sparsipilum* var. *llallaguanianum*, *S. velascanum*, *S. vernei*; series Tuberosa: *S. tuberosum*, Group Stenotomum, Subgroup Goniocalyx, Group Thureja, Group Tuberosum, *S.* × *curtilobum*.

[6] J. D. Mold, R. K. Stevens, R. E. Mean and J. M. Ruth, *Biochemistry* **2**, 605 (1963).

The other species examined gave results which correlate reasonably well with other taxonomic criteria. With one exception, to be discussed in a later section, different clones of the same species gave similar results.

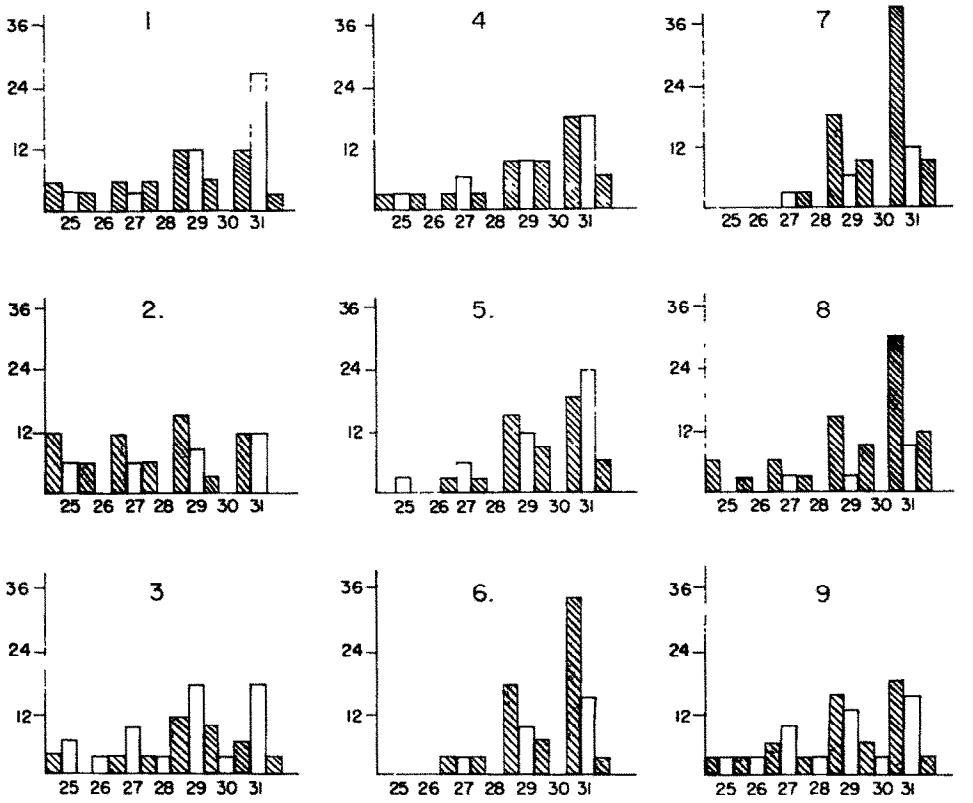

FIG. 2. PROPORTIONATE AMOUNTS OF HYDROCARBONS IN THE C_{25}-C_{32} RANGE, EXTRACTED FROM THE SURFACE WAXES OF FLOWERS OF TUBER-BEARING *Solanum* SPECIES.

Branched constituents are shaded; each immediately precedes its normal homolog. Percentages are to the nearest 3 per cent. In each case the pattern illustrated is that of one individual arbitrarily chosen to represent the pattern type. The name of the individual chosen is listed first in each pattern grouping.

1. *S. tuberosum* "Russet Burbank".
2. *S. tuberosum* "Bliss Triumph" ("Norland", "Dazoc", "Fruhperle", "Blanca", "Bounty").
3. *S. demissum* (*S. stoloniferum* var. *malinchense*).
4. *S. verrucosum*.
5. *S. polytrichon* (*S. trifidum*, *S. clarum*, *S. fendleri*, *S. stoloniferum*).
6. *S. berthaultii* (*S. jamesii*, *S. sambucinum*, *S. tarijense*, *S. ehrenbergii*).
7. *S. pinnatisectum* (*S. chacoense*, *S. commersonii*).
8. *S. polyadenium*.
9. *S. brachycarpum* (*S. guerreroense*, *S. stoloniferum*).

These patterns fell into three major groups, which, in one instance, can be further divided into several sub-groups. Graphs representing these groups and sub-groups are given in Fig. 1, 3–9. In Table 1 are given the chromosome numbers, series assignments,[1] geographic origins, and general fruit and flower morphology of these species.

TABLE 1. SERIES ASSIGNMENTS, P.I. NUMBERS, GEOGRAPHIC ORIGINS, GENERAL FRUIT AND FLOWER MORPHOLOGY, CHROMOSOME NUMBERS, AND NUMBER OF GRAPH REPRESENTATIVE OF HYDROCARBON PATTERNS OBTAINED FROM WILD *Solanum* SPECIES DISCUSSED

Species	P.I. No.	Series*	Chromosome number	Geographic origin	Corolla	Fruit	Graph No.
S. demissum	160227, 161153, 175411	Demissa	$2n=72$	Mexico	Rotate	Globose	3
S. stoloniferum var. *malinchense*	195167	Longipedicellata	$2n=48$	Mexico	Rotate–stellate	Globose	3
S. verrucosum	160228	Demissa	$2n=24$	Mexico	Rotate	Globose to ovoid	4
S. trifidum	255538	Trifida	$2n=24$	Mexico	Stellate	Ovoid–conical	5
S. clarum	243355, 275202, 275205	Clara	$2n=24$	Guatemala	Stellate	Globose	5
S. fendleri	275158	Longipedicellata	$2n=48$	N. America	Rotate–stellate	Globose	5
S. polytrichon	186545, 184772, 184773	Longipedicellata	$2n=48$	Mexico	Rotate–stellate	Globose	5
S. stoloniferum	195195, 205522	Longipedicellata	$2n=48$	Mexico	Rotate–stellate	Globose	5, 9
S. sambucinum	275243	Pinnatisecta	$2n=24$	Mexico	Stellate	Globose	6
S. jamesii	195190, 275171	Pinnatisecta	$2n=24$	N. America	Stellate	Globose	6
S. tarijense	195206	Tarijensa	$2n=24$	Bolivia	Sub-stellate	Globose to ovoid	6
S. berthaultii	218215	Tarijensa	$2n=24$	Argentina	Sub-stellate	Globose to ovoid	6
S. ehrenbergii	251725, 255519	Cardiophylla	$2n=24$	Mexico	Stellate	Globose	6
S. pinnatisectum	184764	Pinnatisecta	$2n=24$	Mexico	Stellate	Globose	7
S. chacoense	133644, 189217, 189220	Commersoniana	$2n=24$	Argentina	Stellate	Globose to ovoid	7
S. commersonii	243503	Commersoniana	$2n=24$	Argentina	Stellate	Globose to ovoid	7
S. polyadenium	275237, 275238	Polyadenia	$2n=24$	Mexico	Sub-stellate	Ovoid	8
S. oxycarpum	230479	Conicibaccata	$2n=48$	Mexico	Rotate–stellate	Long–conical	—
S. hougasii	161174	Demissa	$2n=72$	Mexico	Rotate	Ovoid	—
S. guerreroense	161727, 161730	Demissa	$2n=72$	Mexico	Rotate	Ovoid	9
S. brachycarpum	230459, 243344, 275183	Demissa	$2n=72$	Mexico	Rotate	Ovoid	9

* According to Correll (1962).

Group I

Included in Group I are *S. demissum*, *S. verrucosum*, of series *Demissa*, and *S. stoloniferum* var. *malinchense*, of series Longipedicellata. The *S. demissum* samples, although they showed considerable diversity of hydrocarbon pattern, did have one property in common. In each case, the odd-n peak exceeded the branched peaks on either side of it. This property is shared by only one other North American sample tested, namely *S. stoloniferum* var. *malinchense*. Graph 3 (Fig. 2) is representative of this type. The other *S. stoloniferum* samples tested were quite different. *S. verrucosum*, Graph 4 (Fig. 2), has been included here due to the absence of a dominant peak, a characteristic which clearly distinguished it from all other North American diploids tested.

The proposed origin of the Mexican hexaploids makes these results of interest. *S. demissum*, a hexaploid, is thought to have arisen as a result of crossing of *S. verrucosum*, a diploid, and one of the Longipedicellata tetraploids followed by chromosome doubling.[2, 7, 8] Due to the common occurrence of certain genes[9] in *S. demissum* and *S. stoloniferum*, Dodds[10] has concluded that the Longipedicellata tetraploid involved in the origin of *S. demissum* was *S. stoloniferum*.

However, the immunological studies of Gell, Hawkes and Wright[8] showed no close relationship of *S. stoloniferum* to *S. demissum*. The present study indicates that different clones of this complex polymorphic species are also quite different chemically, a factor which may account for this discrepancy.

Group II

Group II is distinguished by its predominant n-C_{31} peak. It differs from Group I in that at least one branched peak, usually C_{29}, exceeds the normal peak of the same carbon number. Graph 5 (Fig. 2) is representative of this group of Mexican species. Included are *S. trifidum*, the only species in series Trifida; *S. clarum*, the only species in series Clara; and two species found in series Longipedicellata, *S. fendleri* and *S. polytrichon*. One sample of *S. stoloniferum* also falls here.

S. clarum, with its simple leaves, and *S. trifidum*, with a leaf which is often 3-foliate, may be distinguished from the other Mexican species examined on this basis. The tetraploid species in series Longipedicellata are characterized in part by a distinctive rotate-stellate corolla and petiolulate leaflets. The similarity of pattern for the members of series Longipedicellata seems to correlate well with the morphological similarities noted for this series. On the other hand, the similarity of pattern of these species and the otherwise dissimilar *S. trifidum* and *S. clarum* was an unexpected result. Perhaps these results do point to a phylogenetic relationship despite the other differences noted.

Group III

Group III includes those species whose hydrocarbon patterns showed both br-C_{31} and br-C_{29} exceeding the normal peak of the same carbon number. This group further divides into several sub-groups.

Group IIIA contains diploid species, both South American and Mexican, with stellate or sub-stellate corollas. These species often had one very large predominant peak, in the

[7] G. E. K. MARKS, *Genetics* **53**, 262 (1955).
[8] P. G. H. GELL, J. G. HAWKES and S. T. C. WRIGHT, *Proc. Roy. Soc. (London)*, **B151**, 364 (1960).
[9] R. MCKEE, *Euphytica* **11**, 42 (1962).
[10] K. S. DODDS, In *Essays on Crop Plant Evolution* (Edited by Sir J. HUTCHINSON). Cambridge University Press, London (1965).

Mexican species sometimes exceeding 40 per cent of the total. With the exception of the distinctive *S. polyadenium*, none had a peak in the C_{25} or C_{27} sets exceeding 5 per cent of the total.

As can be seen by Fig. 2 (Graphs 6–8), this group can be subdivided still further on the basis of the relative size of the even-numbered branched component to the normal component of one less carbon number. Those in which the branched C_{30} peak was smaller than or equal to the n-C_{29} were the Mexican species *S. jamesii* and *S. sambucinum* of series Pinnatisecta; *S. ehrenbergii* of series Cardiophylla; and the South American species *S. berthaultii* and *S. tarijense*, of series Tarijensa (Graph 6, Fig. 2). Morphologically, *S. ehrenbergii* might well be included in series Pinnatisecta. The species in series Tarijensa are distinguished by their glandular pubescence, a feature which also is characteristic of *S. polyadenium*, discussed below.

Species in which the br-C_{30} exceeds the n-C_{29} are *S. pinnatisectum* of the Mexican series Pinnatisecta, *S. chacoense* and *S. commersonii* of the South American series Commersoniana, all represented by Graph 7, and *S. polyadenium* of the Mexican series Polyadenia, Graph 8 (Fig. 2). As can be seen from the graphs, *S. polyadenium* is distinguished from any other species examined in that the br-C_{28} and br-C_{26} also exceed the n-C_{27} and n-C_{25} respectively. Morphologically, *S. polyadenium* is also quite dissimilar from the other species examined, and is probably the most difficult of the species studied to hybridize with other species.[8,11] The two series Pinnatisecta and Commersoniana are morphologically quite similar, being maintained separately because of the wide geographic separation of the areas where they are now found.

Group IIIB was characterized by a larger proportionate amount of material in the C_{25} and C_{27} sets. The species in this group are Mexican polyploids with rotate corollas. Included are a tetraploid, *S. oxycarpum*, of series Conicibaccata, the rest of the *S. stoloniferum* samples, and the other three Mexican hexaploids of series Demissa examined, *S. hougasii*, *S. guerreroense*, and *S. brachycarpum*. Graph 9 (Fig. 2) is representative of *S. guerreroense*, *S. brachycarpum*, and some *S. stoloniferum* samples. The other two species in this group, *S. hougasii* and *S. oxycarpum*, showed more material in the br-C_{29} than in the br-C_{31} peak.

This result would tend to confirm Correll's opinion[1] that including these hexaploids with *S. demissum* and *S. verrucosum* is not the best arrangement for these species, although the hexaploids in Group III are thought to have shared the same common diploid ancestor, *S. verrucosum*, with *S. demissum*.[5] These data support the proposal that some forms of *S. stoloniferum* may have been the tetraploid ancestors of *S. guerreroense* and *S. brachycarpum*.[7] *S. oxycarpum* appears more probable for this role in the formation of *S. hougasii*.

CONCLUSIONS

In general, the results of this work tend to confirm relationships between species thought to exist on the basis of morphological, cytogenetic, and interfertility data. The patterns found do not seem to be a reflection of the ecological conditions of the areas where these species now occur. In several instances, very similar patterns were found for species which are morphologically quite similar, but are now widely separated geographically. In other cases, sympatric species also gave similar patterns.

In contrast to the hydrocarbon content of most leaf waxes reported in the literature,[3–5] these inflorescence hydrocarbons showed a relatively high proportionate amount of branched

[11] K. K. PANDEY, *Am. J. Botany* **49**, 874 (1962).

constituents, including those of even carbon number, although the even-numbered straight-chain isomers are present in very small amounts, if at all.

Patterns obtained from colchicine-induced polyploids were very similar to those of the parent diploid species. In contrast, the naturally occurring polyploids in general were distinguishable from the diploids, with the exception of *S. verrucosum*, by their lack of one or a few dominant peaks, with more material occurring in the lower carbon number range. The extremely large amount of branched C_{25} constituent in the commercial varieties may be of especial interest.

EXPERIMENTAL

Plant Material

Tubers and/or seeds of forty-two *Solanum* species were obtained from the Inter-Regional Potato Introduction Station, Sturgeon Bay, Wisconsin, and grown in a greenhouse. Tubers harvested from these plants were also planted in a field.

Flowers were collected from thirty-five of these species, dried overnight at 100°, and stored until the chemical tests could be run.

Extraction and Analysis

To each dried flower sample (0·1–1·0 g), 100 ml of chloroform was added in a 500 ml Erlenmeyer flask and the mixture was agitated with a magnetic stirrer for 1 hr. Solids were removed by filtration and the solvent completely removed by vacuum rotary evaporation at about 50°. The residue was dissolved in 1–2 ml of hexane and transferred to a 1 × 11 cm column for separation of the hydrocarbons from the more polar components of the mixture. The column was prepared from a slurry of 4·5 g of Unicel (activated silicic acid) (Clarkson Chemical Co.), in hexane, and washed with 50 ml of hexane before the sample was added. The hydrocarbon fraction was eluted with 100 ml of hexane. The resulting solution was concentrated to 2 ml by vacuum rotary evaporation at about 50 . The remaining solution was transferred to a weighed vial, and evaporation was completed by passing a stream of nitrogen over the vial while it was suspended in a warm water bath. The sample weight was then determined. Sample weights ranged from 0·01 to 0·1 per cent of the dry weight of the inflorescences.

The purity of the hydrocarbons was checked periodically by thin-layer chromatography using standard "silica gel G" (Brinkman Instruments, Inc.) plates with *n*-hexane as solvent in a saturated chamber. Checks for the presence of unsaturation using iodine vapor were negative in every instance.

The samples were next dissolved in sufficient hexane to give a concentration of 100 μg/μl. Two μl of each sample were gas-chromatographed using an F and M Model 400 gas chromatograph fitted with a 1·25 m, 6 mm column containing 3·8% SE30 on Gas Chrom Q, 80–100 mesh. The column temperature was 230 , flow rate of the carrier (Helium) was 60 ml/min. The detector was hydrogen flame. Assignments of homologous series were made on the basis of the linear relationship, under isothermal conditions, between the log of the retention time and the carbon number. The log-plots were verified by the use of standard hydrocarbon samples.

It was assumed that the branched compounds had retention times slightly less than the normal compounds of the same carbon number. However, it is possible that a highly branched compound could appear in the position of a slightly branched compound containing one less carbon.

Care was exercised to avoid contamination from extraneous hydrocarbons. All solvents were redistilled and checked periodically for hydrocarbons in the C_{25} and C_{35} range. The column material was thoroughly washed to remove adsorbed contaminants. The solvents were not allowed to come into contact with plastic (other than Teflon), grease, paraffin or paper. The caps of the sample vials were metal-lined; no stop-cock grease was used on the rotary evaporator joints; filtering was done through glass or porcelain filters without filter paper.

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