Hydrocarbon Composition of Some Crude and Refined Edible Seed Oils*

A. KUKSIS

From the Department of Biochemistry, Queen's University, Kingston, Ontario, Canada

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The hydrocarbon portions (1–10% of the total unsaponifiable matter) of crude corn and wheat germ oils and refined corn, cottonseed, olive, safflower, soybean, and sunflower oils were isolated on silicic acid and analyzed by gas chromatography. All the oils were shown to contain mixtures of both odd and even carbon number saturated hydrocarbons ranging in chain length from C_{13} to C_{35}, but the odd carbon number derivatives were present in somewhat greater proportions. While sunflower, safflower, and olive oils contained mainly the normal homologs of the odd and even series, the others contained in addition significant amounts of nonnormal chain paraffins which were tentatively identified as the mixed *iso-* and the 1-cyclohexyl derivatives. Only olive and wheat germ oils contained large concentrations of unsaturated material, a great proportion of which could be accounted for as squalene. The paraffin portions of crude, refined, and molecularly distilled corn oils had comparable compositions; however the results showed that some losses in the unsaturated material occurred during the refining process. All the hydrocarbon mixtures contained additional amounts of other, as yet unidentified, material.

Small amounts of hydrocarbons have been found in many naturally occurring fats and waxes. Their presence in the seed oils has been attributed (Deuel, 1951) to dissolution of the protective wax coatings of the seed hulls during the oil-isolation process. Despite their minor concentrations, the identity and possible physiological action of these residues on lipid metabolism has become of interest in view of the greatly increased consumption of vegetable oils. Only a few limited studies (Deuel, 1951; Warth, 1957) have been reported regarding the hydrocarbon composition of seed oils or seed waxes.

The following report is an attempt to describe the total hydrocarbon composition of some common-crude and refined edible seed oils. The utilization of a gas chromatographic system capable of an essentially complete resolution of the *normal*, *iso*, and what was believed to be *cyclic* derivatives of odd and even carbon number hydrocarbons has permitted a quantitative estimation of these paraffins without prior fractionation. In addition, the high-efficiency column has provided evidence that all the oils examined also contained other hydrocarbons, including unsaturated and possibly multiple-branched derivatives.

Materials and Methods

Standards.—Purified preparations of C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, and C_{20} saturated normal-chain hydrocarbons were obtained from Applied Science Laboratories, Inc., State College, Pa. The saturated normal-chain C_{28} and C_{32} hydrocarbons were obtained from Fisher Scientific Co., Montreal, Quebec, and were of the Highest Purity grade. The purity of these materials as estimated by gas chromatography was 95–99%.

Source of Unknowns.—Refined soybean, peanut, and cottonseed oils were obtained from Canada Packers Ltd., Toronto, Ontario. Refined safflower, sunflower, and olive oils were supplied by the Pacific Vegetable Oil Corp., Richmond, Calif. Molecular distillates of corn oil were supplied by the Distillation Products Industries, Rochester, N. Y. The refined corn oil (Mazola) and the unsaponifiable matter of crude corn oil were provided by the Corn Products Co., Argo, Ill. The unsaponifiable matter of crude wheat germ oil (Viobin) was purchased from the Viobin Corp., Monticello, Ill.

Preparation of Hydrocarbon Mixtures.—The total hydrocarbon portions from the oils and the commercial unsaponifiable matters were isolated following saponification (100-g portions) and silicic acid chromatography as previously described (Kuksis and Beveridge, 1960). A partial separation of the saturated and the unsaturated hydrocarbons was achieved in a few cases on alumina columns (Merck, acid-washed, 100–200 mesh) by elution with petroleum and benzene, respectively, or by crystallization from acetone. For gas chromatography these materials as well as portions of the total unsaponifiable matter were dissolved in petroleum or carbon disulfide to give solutions of 1–5% (w/v), 1–5 μl of which usually gave peak areas satisfactory for a quantitative analysis.

Methods of Gas Chromatography.—The gas chromatography apparatus consisted of an Aerograph Hy-Fi, Model A-600-B, the F and M Linear Temperature Programmer, Model 40, and a Minneapolis-Honeywell Brown 1-mv recorder equipped with a disc integrator. Nitrogen was the carrier gas. The separations were performed on a stainless steel column 0.3 cm o.d. by 183 cm (1/8 in. by 6 ft), packed with Chromosorb W (60–80 mesh) previously coated with 5% (w/w) SE-30 (Wilkens Instrument and Research, Walnut Creek, Calif.) by the solvent-evaporation technique. The flash-evaporator temperature varied from 250 to 300° depending on the starting temperature of the oven. For a general survey of the hydrocarbon composition of these oils, temperature-programed runs were made from 65 to 270° at about 2.1° per minute. For quantitative estimations stepwise temperature programing was used. Selected periods of temperature programing were alternated with periods of isothermal operation. For maximum resolution of the nonnormal paraffin peaks isothermal runs at selected temperatures were also performed. Under the conditions of uniform temperature programing this column gave a calculated theoretical plate number of 15,000–20,000 (dotriacontane).

For subtractive gas chromatography this column was preceded by a 0.3-cm o.d. by 20-cm (1/8-in. by 8-in.) stainless steel column packed with Linde Molecular Sieve 5A (40–60 mesh) (Downing *et al.*, 1960). In order to minimize tailing, the precolumn was maintained at about the same temperature as the injector block.

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Identification of Individual Components.—The normal-chain hydrocarbons were identified by cochromatography with known standards. Where standards were not available, the identification was based on the relative retention times and the fact that the peaks corresponding to normal-chain paraffins were retained by the molecular sieve. The presence of the mixed *iso* and 1-cyclohexyl derivatives was suggested on the basis of the relative retention times and the correspondence in the characteristic gas chromatographic behavior to well-documented reports in the literature (Levy *et al.*, 1961; Levy and Paul, 1963). The order of the elution of the *iso* and *anteiso* derivatives was assumed to be that described by Downing *et al.* (1960), who used a similar type of stationary phase and noted that the branched derivatives were eluted ahead of the corresponding normal-chain hydrocarbons, and that the *iso* preceded the *anteiso* hydrocarbon. The narrow-bore column employed in the present investigation permitted an essentially complete separation of the mixed *iso* and the cyclohexyl derivatives as well as a partial resolution of the *anteiso* and the *iso* pairs. Plots of retention times (under uniform temperature programing) versus carbon numbers for the *normal*, *iso*, *anteiso*, and the cyclohexyl derivatives gave smooth curves (concave upward), large sections of which approximated perfect straight-line relationships.

Wherever olefins were present, clearly distinguished shoulders preceding the corresponding saturated derivative could usually be seen at low rates of programing. The presence of unsaturated hydrocarbons in a given sample was qualitatively determined by comparing the gas chromatographic elution patterns before and after bromination of the hydrocarbon mixture (Farquhar *et al.*, 1959).

Quantitation of Individual Components and Determination of Recovery.—Using a mixture of equal weights of the saturated normal-chain C_{18}, C_{20}, C_{28}, and C_{32} standard hydrocarbons, it was demonstrated that the area proportions recorded in the hydrogen-flame ionization detector approximated closely those for the weights. The completeness of the recovery of the unknown hydrocarbon mixtures was determined by cochromatography of weighed amounts of the unknown mixture and dotriacontane. Since none of the investigated hydrocarbon mixtures contained more than traces of the C_{32} hydrocarbon, a close agreement between the total weight and area percentages recorded for this hydrocarbon indicated that the unknowns also had been completely recovered. Similar methods were used for determining the amounts of unsaturated material immobilized by bromination. The essential absence of any detectable amounts of hydrocarbons of chain length greater than C_{35} was further confirmed by performing the gas chromatographic runs on the short columns employed for triglyceride chromatography, which had been previously shown (Kuksis and McCarthy, 1962) to permit the recovery of triglycerides of a carbon number of 60.

Results

The total amounts of the mixed hydrocarbons isolated from the various oils are given in Table I. All the refined oils contained less than 0.5% of hydrocarbon material and some contained as little as 0.01%. The values for the total unsaponifiable matter are in the range previously reported for most of these oils (Rosen, 1962). A comparison of the hydrocarbon values for the refined and crude oils as a percentage of total unsaponifiable matter indicates that there have been significant

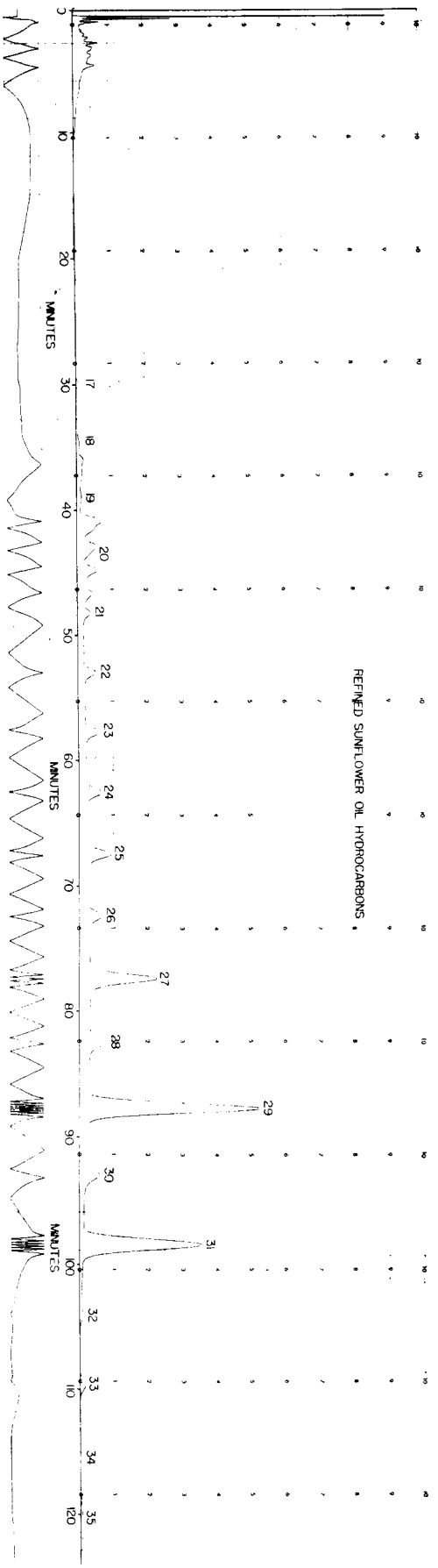

Fig. 1.—Gas chromatogram of refined sunflower oil hydrocarbons. The normal-chain hydrocarbons are identified by their carbon numbers. The sequence of peak at each carbon number is *iso*-paraffin, *normal*-paraffin, 1-cyclohexyl-paraffin of one lower carbon number than the normal paraffin, followed by a repeat of the sequence at the next carbon number.

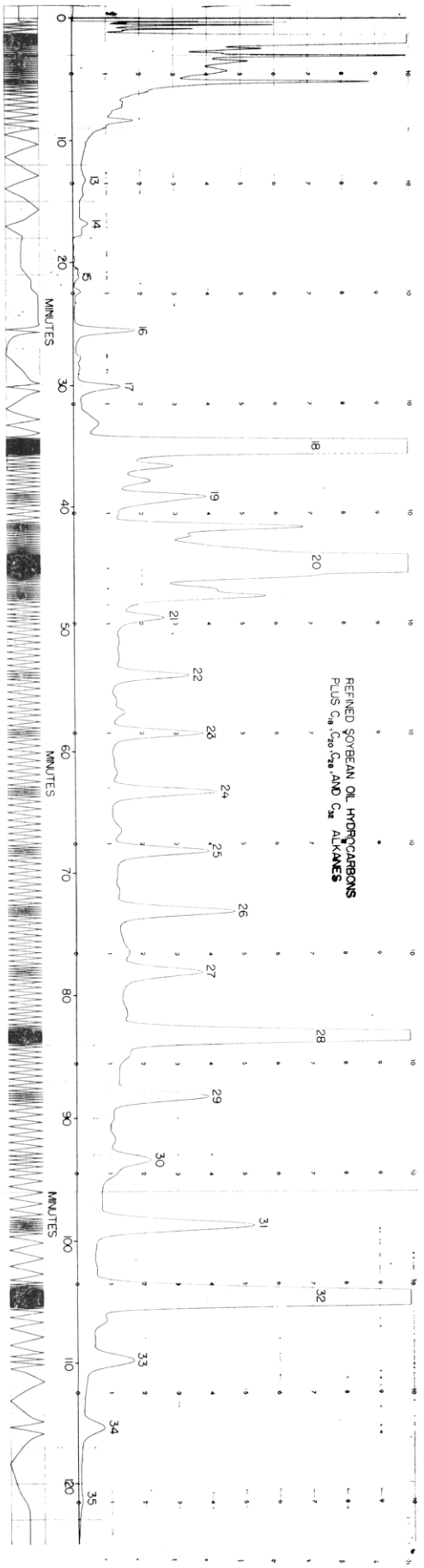

FIG. 2.—Gas chromatogram of refined soybean oil hydrocarbons plus normal-chain C_{18}, C_{20}, C_{28}, and C_{32} alkanes. Legends as in Fig. 1.

TABLE I
TOTAL UNSAPONIFIABLE MATTER AND HYDROCARBON CONTENT OF SOME CRUDE AND REFINED SEED OILS

Original Oil	Total Unsaponifiable Matter (% original oil)	Total Hydrocarbons[a] (% unsaponifiable)	Total Hydrocarbons[a] (% original oil)
Refined corn oil	1.2	2.5	0.03
Refined cottonseed oil	1.0	3.0	0.03
Refined olive oil	0.5	10.5	0.5
Refined safflower oil	0.5	2.0	0.01
Refined sunflower oil	0.3	1.0	Less 0.01
Refined soybean oil	1.1	5.0	0.5
Crude corn oil	1.5	5.0	0.1
Crude wheat germ oil	5.47[b]	1.0	0.05

[a] Material recovered from silicic acid column by petroleum elution. [b] As reported by Drummond *et al.* (1935).

losses in the total amount of hydrocarbon during the refining process (see also Capella *et al.*, 1960).

Figure 1 shows a gas chromatogram obtained for the hydrocarbons of refined sunflower oil. The peaks are well separated and there is little elevation of the base line. A cochromatogram of these hydrocarbons with standards as illustrated for soybean oil in Figure 2 revealed that the major paraffin components of sunflower oil were the normal-chain C_{27}, C_{29}, and C_{31} derivatives. Bromination of the hydrocarbon mixture failed to reveal the presence of any significant amounts of unsaturated material. This was supported by the extreme symmetry of the peaks maintained under conditions of low-increment temperature programing, which was capable of revealing the presence of unsaturated hydrocarbons in other samples (corn oil). Only trace amounts of branched (*iso* and *anteiso*) or "cyclic" derivatives were detected in the sunflower oil either by overloading the column or by subtractive gas chromatography. Readily detectable peaks for the branched and cyclic derivatives could be demonstrated only in the range where the mixed *iso* C_{20} and C_{21}, and the cyclic C_{19} and C_{20} hydrocarbons would be anticipated. The estimated quantitative compositions for the hydrocarbon mixtures from this and other oils are given in Table II.

The hydrocarbons of refined soybean oil (Figs. 2 and 3) are more complex than those of sunflower oil. Major contributions are made by both odd- and even-carbon-number normal paraffins of C_{21} to C_{33}. The C_{31} derivative is again a major component. In addition, unresolved paraffinic materials are also present, bringing about a significant elevation of the base line. The contributions made by the peaks for the *iso* and the cyclic derivatives are minor in comparison to the total amount of the ill-defined material contributing to the base-line elevation. Prolonged gas chromatographic runs indicated that the latter material also moved in discrete bands, but that under the survey conditions it partially overlapped with or was eluted between the peaks of the identified components. The fact that these peaks were not retained by the molecular sieve suggested that they represented other branched or cyclic materials, among which the various methyl or multiple-branched derivatives may have made significant contributions. The contributions of the mixed *iso* and the cyclic derivatives varied from the odd- to the even-carbon-number compounds, but the cyclic derivatives were present in a somewhat greater proportion in the even-carbon-number series.

The peaks with the retention times characteristic

of the *iso* and cyclic C_{19}, C_{20}, and C_{21} compounds were apparently contaminated with some unsaturated material, as a noticeable reduction in the peak size took place following bromination and either a direct or subtractive gas chromatography.

Both *iso* and cyclic derivatives of odd- and even-carbon-number paraffins were present in considerable amounts in the corn oil sample (Fig. 4). The presence of these branched and cyclic derivatives was demonstrated by subtractive gas chromatography when pairs of peaks with the anticipated spacings were readily detected. The normal-chain odd-carbon-number derivatives were present in greater amounts than the normal-chain even-carbon-number paraffins. The C_{21} derivative was the major component with only modest contributions being made by the C_{29} and C_{31} hydrocarbons, which were the major components in the hydrocarbon mixtures from sunflower and soybean oils. In addition, the corn oil hydrocarbons contained significant amounts of unsaturated olefins, which could be clearly seen forming the front part of the normal-chain C_{17}, C_{18}, and C_{19} hydrocarbon peaks. Their unsaturated character was indicated by the results of bromination studies. For quantitative analysis of this sample stepwise temperature programing was used to extend the run and to bring the base line as close to the zero line as possible. As in the case of soybean oil, only the contributions of *normal*, mixed *iso*, and 1-cyclohexyl derivatives are included in Table II. The proportional hydrocarbon composition of the crude corn oil sample was closely similar to that for the refined oil, indicating nonselective paraffin losses during the refining process.

The hydrocarbon composition of the safflower oil sample, represented in Figure 5, is similar to that for sunflower oil. The odd-carbon-number normal chains, C_{27}, C_{29}, and C_{31}, make the major contributions. However, in contrast to sunflower oil, this oil also contains significant amounts of other odd- and even-carbon-number normal, *iso*, and particularly the "1-cyclohexyl" chain materials. The branched-chain peaks in the C_{20} and C_{21} region again showed signs of unsaturation and were probably other than the *iso* or the "cyclic" derivatives of the C_{20} and C_{21} and of the C_{18} and C_{19} hydrocarbons, respectively.

Figure 6 represents the total hydrocarbon composition of refined olive oil. Some 75% of the total hydrocarbon was made up of squalene, which, although possessing a 30-carbon skeleton, moved with the C_{28} derivatives because of its more compact structure. The remaining 25% of the material was made up of varying proportions of all the odd- and even-carbon-number straight-chain derivatives from C_{17} to C_{34}. The part of the chromatogram anticipated to be occupied by the mixed *iso* and the cyclic derivatives of C_{19}, C_{20}, and C_{21} hydrocarbons was again contaminated with unsaturated material, which contributed particularly heavily to the peak for the *iso* derivative of C_{20}. In order to obtain reliable quantitative estimates for the C_{27}, C_{28}, and C_{29} hydrocarbons, the squalene peak was removed by silicic acid chromatography.

Of the oils examined, wheat germ oil was the only other oil which contained significant amounts (about 50%) of squalene in its hydrocarbon mixture. It also contained proportionally large quantities of unsaturated material with retention times similar to those anticipated for the *iso* and the 1-cyclohexyl members of the C_{19} and C_{20} hydrocarbons. The wheat germ oil contained larger quantities of the even-carbon-number normal-chain derivatives than the other oils (Table II). Although the major peak was again given by the normal-

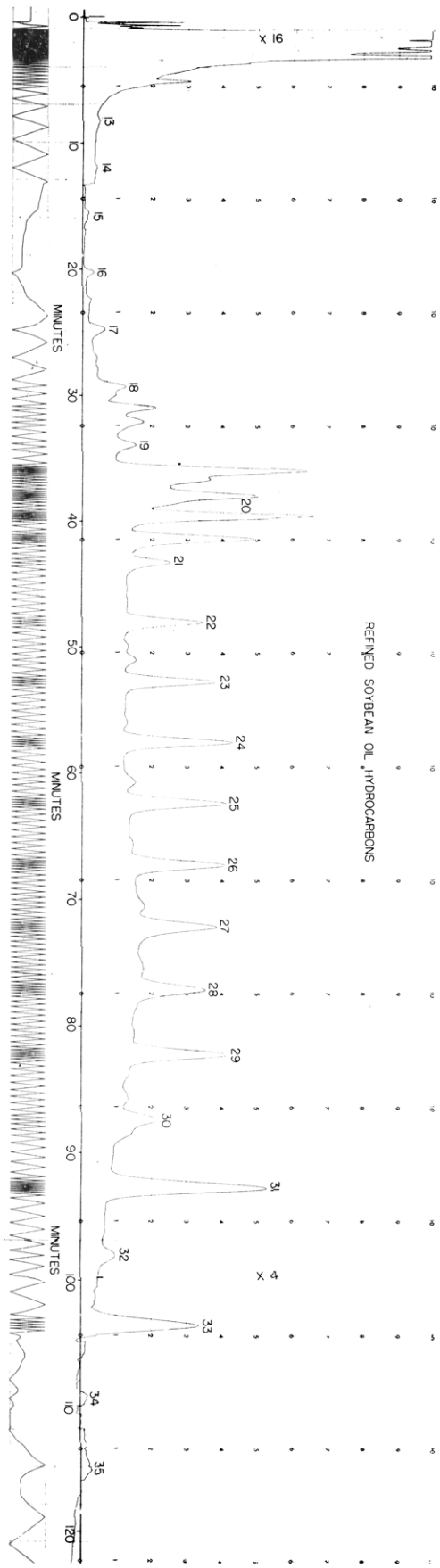

FIG. 3.—Gas chromatogram of refined soybean oil hydrocarbons. Legends as in Fig. 1.

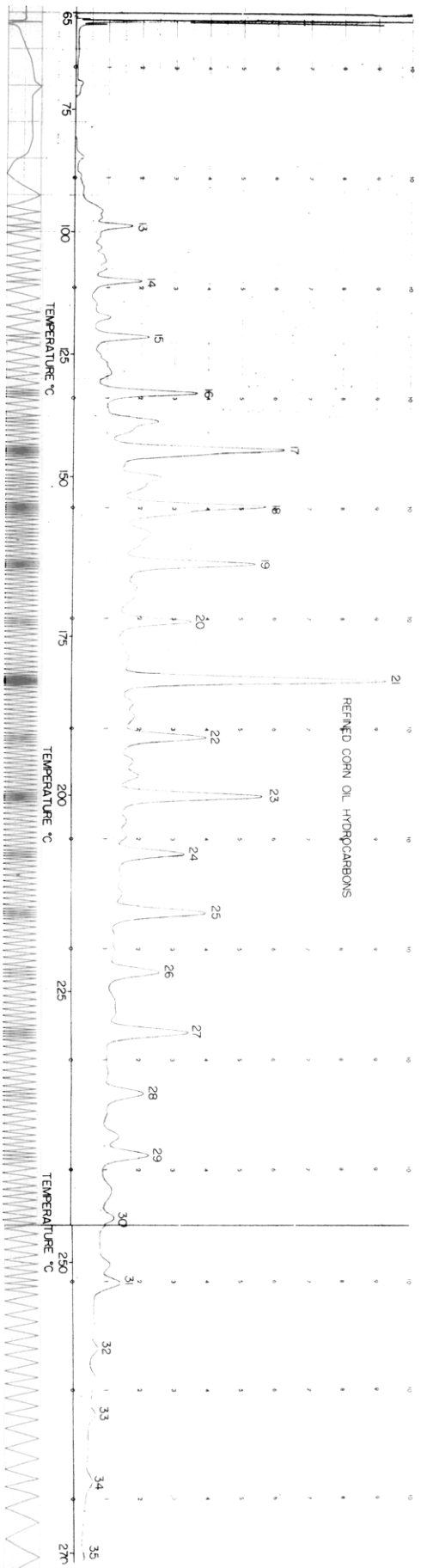

FIG. 4.—Gas chromatogram of refined corn oil hydrocarbons. Legends as in Fig. 1.

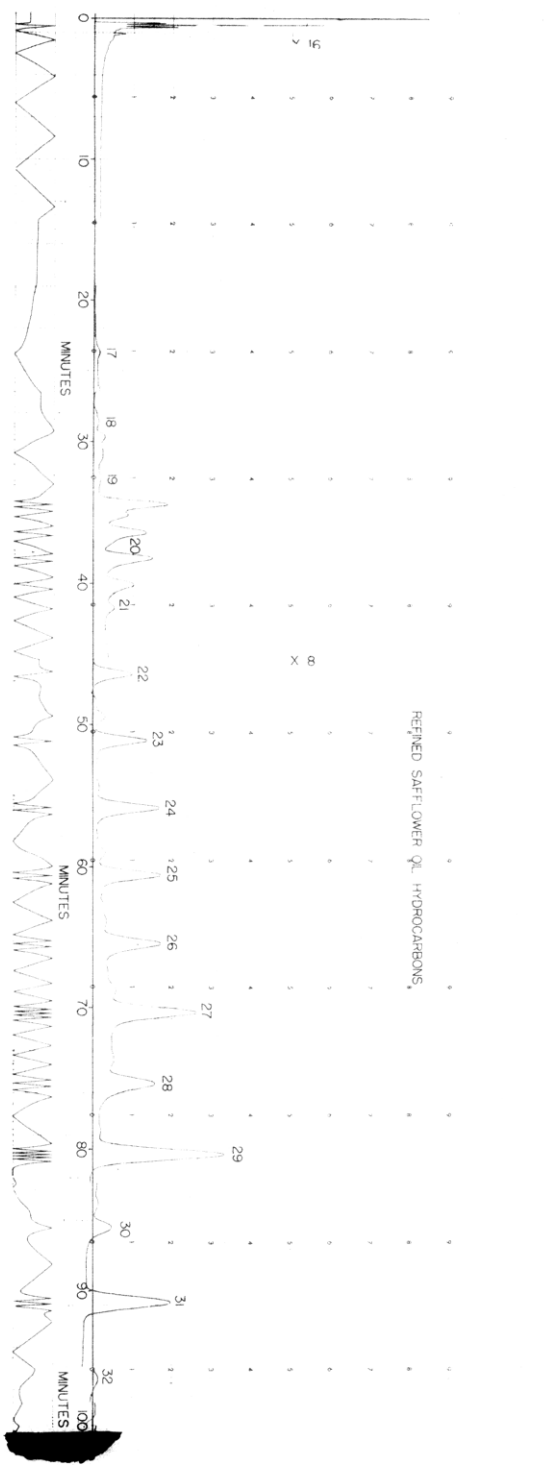

FIG. 5.—Gas chromatogram of refined safflower oil hydrocarbons. Legends as in Fig. 1.

chain C_{29} hydrocarbon, the normal-chain C_{25} and C_{27} members were present in only traces.

The hydrocarbon mixture of refined cottonseed oil resembled that of corn oil since it also contained the *n* C_{21} hydrocarbon as the major (31%) component. However, there was more of the longer-chain material present in the cottonseed oil hydrocarbon mixture, the difference being made up by a near absence of the C_{23} to C_{28} hydrocarbons. In contrast to some of the other oils, cottonseed oil contained considerable quantities of the cyclic C_{36} (6%) and *iso* C_{32} (6%) hydrocarbons. It was poor in the branched-chain and cyclic isomers of the lower homologs.

TABLE II

HYDROCARBON COMPOSITION OF SOME CRUDE AND REFINED EDIBLE SEED OILS

Hydrocarbon[a]	Crude Corn Oil	Crude Wheat Germ Oil	Refined Soybean Oil	Refined Sunflower Oil	Refined Safflower Oil	Refined Olive Oil	Refined Cottonseed Oil
n C_{13}	1.2		Trace[b]				
n C_{14}	1.8		Trace				
c C_{13}	0.1						
i C_{15}	0.3						
n C_{15}	2.4		Trace				
c C_{14}	0.1						
i C_{16}	0.3						
n C_{16}	4.8		0.2				0.5
c C_{15}	0.8						Trace
i C_{17}	0.5						0.5
n C_{17}	8.0	9.2	0.5	Trace	Trace	2.4	0.8
c C_{16}	0.8	Trace					2.0
i C_{18}	0.4	Trace					Trace
n C_{18}	7.2	Trace	0.6	Trace	Trace	0.1	Trace
c C_{17}	0.7	7.9	1.8[c]			0.1	0.5
i C_{19}	0.5	16.2[c]	1.7			0.1	8.5[c]
n C_{19}	7.2	2.1	1.7	Trace	Trace	0.2	0.5
c C_{18}	0.3	2.1	6.5[c]	5.1[c]	9.5[c]	0.7	0.8
i C_{20}	0.3	0.6	8.3[c]	2.6	4.5	7.0[c]	Trace
n C_{20}	3.3	2.8	1.8	1.0	Trace	0.7	Trace
c C_{19}	0.1	0.6	8.0	2.6	5.0	0.9	1.1
i C_{21}	0.1	Trace	5.9	1.3	3.8	0.7	0.8
n C_{21}	14.5	4.7	2.2	1.1	Trace	0.1	31.0
c C_{20}	0.3	Trace					12.4
i C_{22}	0.3	0.6					1.1
n C_{22}	5.7	9.7	3.9	2.4	3.8	0.5	2.3
c C_{21}	0.3	0.6					Trace
i C_{23}	0.4	0.6					Trace
n C_{23}	8.0	2.8	4.8	2.7	5.0	0.9	0.5
c C_{22}	0.2	Trace					Trace
i C_{24}	0.2	0.6					Trace
n C_{24}	3.3	5.5	5.6	2.9	6.4	0.9	0.8
c C_{23}	Trace	Trace					Trace
i C_{25}	Trace	Trace					Trace
n C_{25}	5.9	Trace	5.6	3.1	7.2	1.0	0.5
c C_{24}	Trace	Trace					0.5
i C_{26}	Trace	0.6					Trace
n C_{26}	3.3	4.7	5.0	2.9	8.0	0.9	Trace
c C_{25}	0.1	Trace					Trace
i C_{27}	0.1	Trace					Trace
n C_{27}	5.0	0.6	4.9	10.7	10.8	0.9	Trace
c C_{26}	Trace	Trace					Trace
i C_{28}	Trace	Trace					Trace
n C_{28}	3.0	2.3	4.8	3.1	6.5	78.0	1.1
c C_{27}	Trace	Trace					0.5
i C_{29}	0.5	Trace					Trace
n C_{29}	3.1	16.4	7.5	32.1	15.9	2.4	7.4
c C_{28}		Trace					Trace
i C_{30}	0.5	Trace					6.6
n C_{30}	0.5	2.8	3.0	3.1	2.9	0.3	6.2
c C_{29}	Trace	Trace					1.5
i C_{31}	0.4	Trace					Trace
n C_{31}	1.9	3.2	11.8	21.9	10.1	0.9	0.8
c C_{30}	Trace	Trace					6.2
i C_{32}	Trace	Trace					Trace
n C_{32}	0.4	0.6	1.3	0.5	0.6	Trace	0.8
c C_{31}		Trace					
i C_{33}	0.3	Trace					
n C_{33}	0.4	1.1	2.4	1.1	Trace	0.1	1.5
c C_{32}	Trace						
i C_{34}	Trace						1.1
n C_{34}	0.4	Trace	Trace	Trace	Trace	Trace	0.8
c C_{33}	Trace						
i C_{35}	Trace						
n C_{35}	Trace	Trace	Trace	Trace	Trace	Trace	0.8

[a] *n* = normal chain; *i* = mixed *iso* and *anteiso* chains; *c* = 1-cyclohexyl chain. [b] These peaks were detectable but constituted less than 0.1% of the total. [c] These peaks were composed of saturated and unsaturated material. The results given include both peaks.

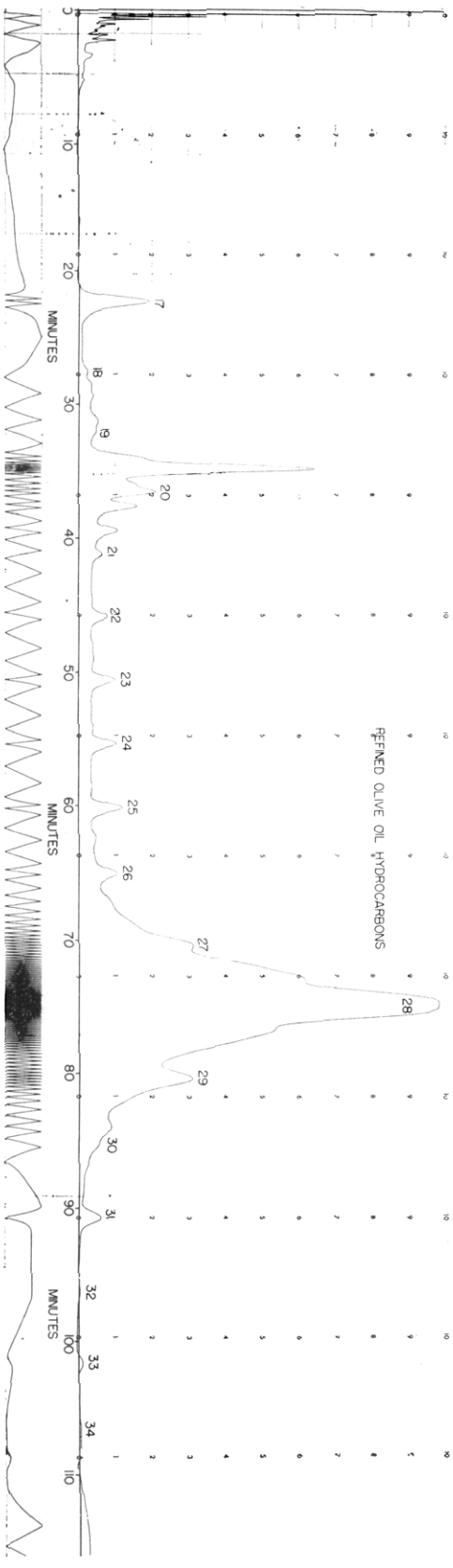

FIG. 6.—Gas chromatogram of refined olive oil hydrocarbons. Legends as in Fig. 1.

DISCUSSION

In regard to the occurrence of the unsaturated hydrocarbon gadusene in soybean (Nakamiya, 1935) and wheat germ oils (Drummond *et al.*, 1935), the present study could provide no evidence. Both these oils were low in the C_{18} hydrocarbons (Table II). Wheat germ oil, and to a lesser extent soybean oil, did, however, contain unsaturated components that had retention times corresponding to those for the cyclic C_{18} and the *iso* C_{19} derivatives. Similarly, the findings of Marcelet 1936a,b) concerning the presence of the unsaturated C_{13}, C_{16}, C_{19}, C_{23}, and C_{28} hydrocarbons in olive oil remain unsupported by the present data. In fact, no measurable peaks were found for the C_{13}, C_{16}, and C_{19} hydrocarbons, and those for C_{23} and C_{28} were shown to be largely made up of saturated hydrocarbons. There was a peak for the saturated C_{24} normal-chain hydrocarbon which Marcelet also reported. If any of these plant oils contain such unsaturated hydrocarbons, they must have been lost completely in the refining process. Otherwise they have to be dismissed as possible artifacts of steam distillation and the deodorization process. In this connection it may be of interest to note that the unsaturated hydrocarbon melene, supposedly a common constituent of beeswax (Warth, 1956), could not be detected by gas chromatographic methods (Downing *et al.*, 1961).

The amounts of squalene found in the olive and wheat germ oils may be low, as no precautions were taken during the saponification to prevent the destruction of this material. This may also explain why no squalene was found in the corn oil preparation which previously had shown traces of it (Kuksis and Beveridge, 1960). Gas chromatography of the total unsaponifiable matter similarly failed to disclose the presence of specific peaks that could be attributed to carotenes. Whether or not both squalene and the carotenes contributed to the ill-defined unsaturated material eluted in the C_{19} to C_{20} range remains to be established.

Assuming that the paraffins of the edible oils originated in the plant waxes, some similarity in the two would be anticipated. The normal-chain saturated C_{29} and C_{31} hydrocarbons, listed by Warth (1957) to be present in the seed wax of corn, were also found in the corn oil, as were the C_{30}, C_{31}, and C_{33} cotton-wax hydrocarbons in cottonseed oil. They were not, however, either the only or even the major components. A comparison of the data in Table II with the quantitative hydrocarbon compositions determined by gas chromatography for sugar-cane wax (Kranz *et al.*, 1960), carnauba wax (Downing *et al.*, 1961), and tobacco-leaf wax (Mold *et al.*, 1963) indicates that the presently examined oils contain considerably more of the even-carbon number hydrocarbons. With the exception of sunflower oil, which contained large proportions of the C_{27}, C_{29}, and C_{31} paraffins, both odd and even series were approximately equally represented. In difference to the stem wax of *Leptochloa* (Kranz *et al.*, 1961), the present hydrocarbon mixtures were free from any readily detectable long-chain (C_{62} or more) derivatives. If present in the seed waxes, they might have been anticipated to be lost during the processes of oil extraction or refining.

The tentative identification of cyclohexyl hydrocarbons in these plant-paraffin mixtures is most interesting, although their identification in petroleum wax might have been taken as an indication of a possible biological origin. In view of the great differences between the hydrocarbon populations of the paraffin waxes and the seed oils, a contamination of the latter with the former during the refining or isolation processes appears unlikely. Although a more rigid and preferably a direct examination of the seed-wax hydrocarbons would be desirable, the hydrocarbon compositions of the edible oils presented in this paper illustrate the multiplicity of forms and the widespread nature of the occurrence of paraffin residues in these food staples.

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Effect of Androgens on Steroid C-21 Hydroxylation*

DINESH C. SHARMA AND RALPH I. DORFMAN

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C-21 hydroxylation of progesterone, 17α-hydroxyprogesterone, pregnenolone, and 17α-hydroxypregnenolone was studied using the microsomal fraction of bovine adrenal cortex as the source of "steroid C-21 hydroxylase." 4-Androstene-3,17-dione, testosterone, and dehydroepiandrosterone do not ihhibit C-21 hydroxylation of progesterone and 17α-hydroxyprogesterone in contrast to the inhibitory action of the adrenal androgens on 11β-hydroxylation step in corticosteroid biosynthesis. However, C-21 hydroxylation of pregnenolone and 17α-hydroxypregnenolone was markedly inhibited by 4-androstene-3,17-dione, testosterone, and dehydroepiandrosterone. This is of particular significance since 17α-hydroxypregnenolone and not 17α-hydroxyprogesterone has been suggested to be the primary precursor of cortisol, and intraglandular modulation of steroid biosynthesis at least in part correlates certain clinical findings. Effect of pharmacological inhibitors 1,2-bis-(3-pyridyl-2-methyl-1-propanone), 3-(6-chloro-3-methyl-2-indenyl)pyridine, 3-(1,2,3,4-tetrahydro-1-oxo-2-naphthyl)pyridine, and the 7-chloro derivative of 3-(1,2,3,4-tetrahydro-1-oxo-2-naphthyl)pyridine on steroid C-21 hydroxylation has also been reported.

Intraglandular modulation of steroid biosynthesis and its possible role in physiological, and even more in pathological, conditions has recently been postulated (Mahajan and Samuels, 1962; Leventhal and Scommegna, 1963). The influence of certain steroids on 11β-hydroxylation of 11-deoxycorticosterone[1] has been reported earlier (Sharma *et al.*, 1963). In the present paper, steroid C-21 hydroxylation of various substrates has been studied and the effect of certain steroids, particularly adrenal androgens, has been investigated. The effect of certain pharmacological inhibitors has also been reported.

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[1] The following trivial names and abbreviations have been used in this work: progesterone, 4-pregnene-3,20-dione; 17α-OH-progesterone, 4-pregnen-17α-ol-3,20-dione; pregnenolone, 5-pregnen-3β-ol-20-one; 17α-OH-pregnenolone, 5-pregnene-3β,17α-diol-20-one; 11-deoxycorticosterone, 4-pregnen-21-ol-3,20-dione; 11-deoxycortisol, 4-pregnene-17α-21-diol-3,20-dione; testosterone, 4-androsten-17β-ol-3-one; dehydroepiandrosterone, 5-androsten-3β-ol-17-one; G-6-P, glucose-6-phosphate; SU 4885, 1,2-bis-(3-pyridyl-2-methyl-1-propanone); SU 8000, 3-(6-chloro-3-methyl-2-indenyl)pyridine; SU 9055 3-(1,2,3,4-tetrahydro-1-oxo-2-naphthyl)pyridine; SU 10603, 7-chloro derivative of SU 9055.

EXPERIMENTAL PROCEDURE

Triphosphopyridine nucleotide, "98% pure," as the sodium salt and glucose-6-phosphate also as the sodium salt were obtained from Sigma Chemical Co. Glucose-6-phosphate dehydrogenase was obtained from Calbiochem, Inc. SU 8000, SU 9055, and SU 10603 were generous gifts from Dr. J. J. Chart, Ciba Pharmaceutical Co., Summit, N. J. Amphenone, steroids, and all other chemicals were obtained commercially.

Enzyme Preparation.—Bovine adrenal glands were collected within 15 minutes after slaughter, freed from connective tissues and adhering fat, dipped in ice-cold 0.25 M sucrose solution, and placed in dry ice. The frozen glands were stored at −20° and processed within 8 weeks.

The following operations were carried in a cold room at 4°. The cortex was removed while still frozen and was homogenized with three times its weight of ice-cold 0.25 M sucrose solution first in a Waring Blendor at 1/4 speed for 2 minutes and then in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 700 × *g* for 15 minutes at 0° to remove nuclei and cell debris; the supernatant fluid was then centrifuged at 7000 × *g* for 30 minutes to remove the mitochondrial