

# High Resolution Infrared Spectra of Steroids in the Carbon-Hydrogen Stretching Region

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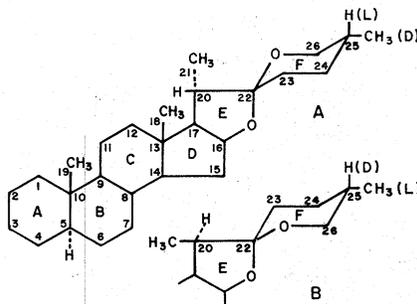
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► The infrared spectra of 40 steroidal sapogenins were investigated in the 3100- to 2750-cm.<sup>-1</sup> region using a double monochromator equipped with lithium fluoride prisms, in order to correlate absorption in the C—H stretching region with structural features of the molecules. In addition to bands which can be assigned to

stretching modes of =CH, —CH<sub>2</sub>—, and —CH<sub>3</sub> groups, absorption maxima and shoulders occur which cannot be easily assigned to normal modes of isolated units. The majority of the observed bands are very sensitive to structural changes in the carbon skeleton. It is frequently possible to distinguish between 25D and 25L configurations, detect opening of E and F rings, and determine the configuration of the methyl group in the 20 position. A detailed study of the C—H region of steroids can thus serve as a useful supplement to investigations at lower frequencies.

INTEREST in the carbon-hydrogen stretching region has increased with the advent of high resolution spectrophotometers. Fox and Martin (1-3) used a grating spectrometer to establish characteristic C—H stretching frequencies for a carefully selected group of hydrocarbons in which one or two functional groups predominated. A similar study on a series of deuterated ketones and esters was conducted by Nolin and Jones (9-11). The Fox and Martin assignments have been confirmed by work on fatty acids (4), heterocyclic nitrogen compounds (15), and oxygen- and sulfur-containing materials (12), and also have been applied to the analysis of mixtures (13, 14). Jones and coworkers (5-7) have discussed the C—H stretching modes in steroids and used them to detect ethylenic double bonds, but no systematic study of the 3000-cm.<sup>-1</sup> region has been reported for steroids.

A large number of reference grade steroidal sapogenins have been prepared at this laboratory by Wall and coworkers (17-19) in the course of a search



Typical skeleton structures of steroidal sapogenins

A. 20 $\alpha$ , 25D sapogenins (3-deoxytigogenin)  
B. 20 $\beta$ , 25L sapogenins

for cortisone precursors. Lithium fluoride spectra were obtained for these compounds to correlate observed bands with molecular structure and to see if the Fox and Martin hydrocarbon assignments could be applied to steroids.

The basic structure of the spirostane ring system of sapogenins has been well established by Marker and others (8). Convincing evidence has been presented (16) for the side-chain stereochemistry of 20 $\alpha$ , 25D sapogenins, illustrated by 3-deoxytigogenin (20 $\alpha$ , 25D allospirostane, Formula A), and for the analogous 20 $\alpha$ , 25L and 20 $\beta$ , 25D compounds (20).

The side-chain structure of the

20 $\beta$ , 25L sapogenins is less firmly established, but is believed (16) to involve ring inversion (Formula B).

Other variations from the illustrated structures include acetylation at C<sub>3</sub> or at C<sub>2</sub> and C<sub>3</sub>, unsaturation at C<sub>5</sub>, introduction of a carbonyl group at C<sub>12</sub>, cis or trans fusion of the A/B-rings, and rupture or cleavage of the E and F rings. Structural differences among the studied sapogenins are listed in Table I. The compounds fall into six general classifications: sapogenins, deoxysapogenins, 20 $\alpha$  sapogenin acetates, 20 $\beta$  sapogenin acetates, open F-ring compounds, and cholestane derivatives.

## EXPERIMENTAL

The infrared absorption studies were carried out on a Beckman Model IR-3 double-monochromator spectrophotometer equipped with lithium fluoride prisms. Spectra were recorded in carbon tetrachloride solution at concentrations of 3 to 10 grams per liter in a 1.02-mm. cell, using a tape-recorded blank of carbon tetrachloride in the same cell as a reference. The mechanical slit width was set at 0.18 mm. at 2750 cm.<sup>-1</sup> and attained a value of 0.15 mm. at 3100 cm.<sup>-1</sup> The spectral resolution of the instrument under these conditions is estimated to be around 3 cm.<sup>-1</sup> (Splitting of HCl<sup>36</sup> and HCl<sup>37</sup> doublets with a separation of about 2 cm.<sup>-1</sup> can be well observed with 0.11-mm. slits; the two bands are just

Table I. Structural Features of Steroidal Sapogenins Studied

Sapogenin	A/B	C <sub>25</sub>	Position of —OCOCH <sub>3</sub> and/or OH	Other Functional Groups
Sarsasapogenin	cis	L	3 $\beta$	
Yamogenin	...	L	3 $\beta$	$\Delta^5$
Tigogenin	trans	D	3 $\beta$	
Diosgenin	...	D	3 $\beta$	$\Delta^5$
Hecogenin	trans	D	3 $\beta$	12 C=O
Gentrogenin	...	D	3 $\beta$	$\Delta^5$ , 12 C=O
Correllogenin	...	L	3 $\beta$	$\Delta^5$ , 12 C=O
Gitogenin	trans	D	2 $\alpha$ , 3 $\beta$	
Yuccagenin	...	D	2 $\alpha$ , 3 $\beta$	$\Delta^5$
Manogenin	trans	D	2 $\alpha$ , 3 $\beta$	12 C=O
Kammogenin	...	D	2 $\alpha$ , 3 $\beta$	$\Delta^5$ , 12 C=O
Chlorogenin	trans	D	3 $\beta$ , 6 $\alpha$	
Markogenin	cis	L	2 $\beta$ , 3 $\beta$	
Smilagenin	cis	D	3 $\beta$	
Rockogenin	trans	D	3 $\beta$ , 12 $\beta$	

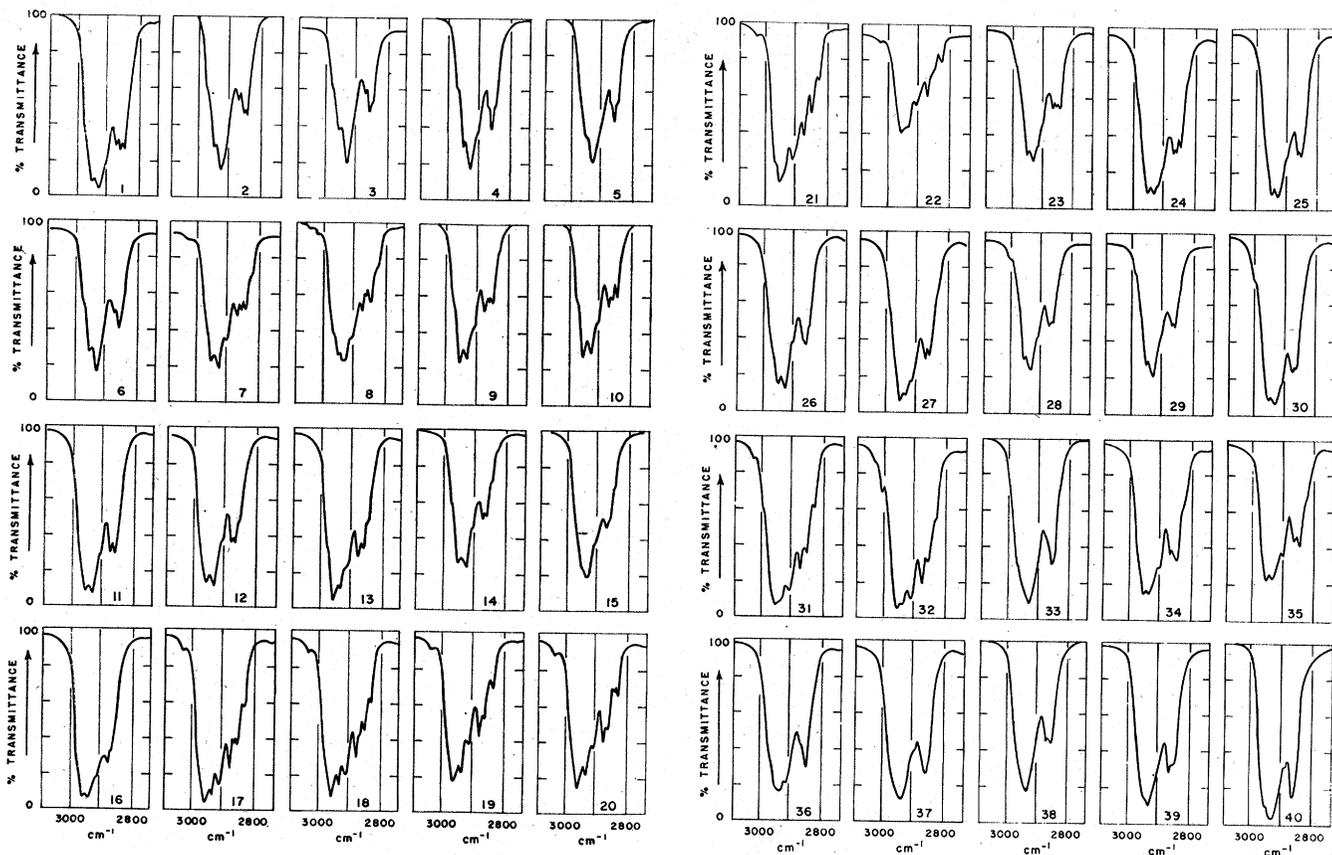


Figure 1. Infrared spectra of steroidal sapogenins and related material in the 3100- to 2750- $\text{cm}^{-1}$  region. See Table II for identification of individual spectra

barely distinguishable with 0.15-mm. slits.)

The isolation and purification of the compounds have been described (17-19). Their high purity was confirmed by melting point, carbon and hydrogen determination, and optical rotation, in conjunction with sodium chloride spectra in the 1800- to 650- $\text{cm}^{-1}$  region. Pure samples of cholestane and coprostane were supplied by Louis Fieser of Harvard University.

#### RESULTS AND DISCUSSION

Fox and Martin and subsequent investigators found one band which they

associated with the  $=\text{CH}$  group and two bands associated with the symmetric and asymmetric stretching of the  $\text{CH}_2$  group. The  $\text{CH}_3$  group gives rise to two main bands assigned to the symmetric and asymmetric stretching modes and to two weaker bands. A weak band, seldom observed, was associated with  $-\text{CH}$  groups. Between seven and

nine bands were found for steroidal sapogenins in the 3100- to 2750- $\text{cm}^{-1}$  region. Assignments were made on the basis of the observations of Fox and Martin and spectral differences produced by changes in molecular structure. Frequencies mentioned in the text are only approximate. Precise

values are listed in Table II. The spectra of the sapogenins in the 3100- to 2750- $\text{cm}^{-1}$  region are shown in Figure 1.

**3035- $\text{Cm}^{-1}$  Region.** A weak band was found around 3035  $\text{cm}^{-1}$  for all the  $\Delta^5$  sapogenins. The band has been

assigned to the  $=\text{CH}$  stretching vibration by Jones and coworkers (5-7). The frequency is 10 to 20  $\text{cm}^{-1}$  higher in the sapogenins than in unsaturated aliphatic hydrocarbons (8).

**3000- to 2960- $\text{Cm}^{-1}$  Region.** Most  $20\beta$ ,  $25\text{D}$  sapogenin acetates exhibit an inflection around 2990  $\text{cm}^{-1}$ ; the  $20\beta$ ,  $25\text{L}$  compounds absorb above 3000  $\text{cm}^{-1}$ . Compounds with a 12-ketone group do not show either of these bands. A shoulder around 2975  $\text{cm}^{-1}$  was found for most  $20\alpha$ ,  $25\text{D}$  sapogenins, but in none of the  $20\alpha$ ,  $25\text{L}$  compounds. In  $\Delta^5$  compounds the shoulder is hardly noticeable or is non-existent. Derivatives of yamogenin (a  $\Delta^5$ ,  $25\text{L}$  sapogenin) show a strong shoulder at 2964  $\text{cm}^{-1}$ . The origin of these shoulders is unknown.

**2950- $\text{Cm}^{-1}$  Region.** All the compounds studied had a band close to 2950  $\text{cm}^{-1}$ , which was attributed to the asymmetrical  $\text{CH}_3$  stretching. The band increases in intensity relative to the neighboring 2930- $\text{cm}^{-1}$  band as the ratio of  $\text{CH}_3$  to  $\text{CH}_2$  groups becomes greater. This ratio is one to three in a typical sapogenin (Formula A). Com-

pounds with open E and F rings show only a weak band in this region. Addition of acetate groups increases the absorption of the 2950- $\text{cm}^{-1}$  band relative to 2930  $\text{cm}^{-1}$ , but the only compound in which  $\text{CH}_3$  absorption exceeds  $\text{CH}_2$  in this region are  $20\alpha$  tigogenin derivatives, and some compounds without a  $\text{C}_6$  methylene group—i.e.,  $\Delta^5$  acetates and the 3,6-diacetate of chlorogenin.

**2930- $\text{Cm}^{-1}$  Region.** Fox and Martin found both  $\text{CH}_3$  and  $\text{CH}_2$  absorption near 2930  $\text{cm}^{-1}$ , but later researchers were unable to resolve the two bands. In the steroid series the band found around 2930  $\text{cm}^{-1}$  was assigned to asymmetrical  $\text{CH}_2$  stretching when comparison of sapogenin acetates with the corresponding deoxy compounds revealed a decrease in absorption due to substitution at the 3 position. The similar effect of  $\text{C}_3$ ,  $\text{C}_6$  acetylation was mentioned above. Loss of an additional methylene group at  $\text{C}_2$  by acetylation does not produce the same effect.

**2920- to 2900- $\text{Cm}^{-1}$  Region.** A shoulder near 2910  $\text{cm}^{-1}$  occurring in most sapogenins is more pronounced in  $\Delta^5$  compounds, and becomes a strong band in  $\Delta^5$  acetates. Its relative intensity varies inversely with that of the 2860- $\text{cm}^{-1}$  band in  $\Delta^5$  acetates. Fox and Martin associated a band in this region with the methyl group in

Table II. Absorption Bands of Steroidal Sapogenins and Related Compounds

Compound	3035 Cm. <sup>-1</sup> =CH	3000-2960 Cm. <sup>-1</sup>	2950 Cm. <sup>-1</sup> Asym. -CH <sub>3</sub>	2930 Cm. <sup>-1</sup> Asym. -CH <sub>2</sub>	2920-2910 Cm. <sup>-1</sup>	2870 Cm. <sup>-1</sup> Sym. -CH <sub>3</sub>	2860 Cm. <sup>-1</sup> Sym. -CH <sub>2</sub>	2850 Cm. <sup>-1</sup>	2830 Cm. <sup>-1</sup>	Spec- trum No., Fig. 1	Concn., G./L.
<b>Sapogenins</b>											
Tigogenin		2978 <sup>a</sup>	2952	2930	2907 <sup>a</sup>	2873	2861	2849		1	7.5
<b>20<math>\alpha</math> 3-deoxysapogenins</b>											
Tigogenin		2973 <sup>a</sup>	2952	2928		2872	2857	2846		2	4.0
Hecogenin			2953	2929		2874	2859	2850 <sup>a</sup>		3	4.3
Smilagenin		2968 <sup>a</sup>	2952	2928		2870 <sup>a</sup>	2862	2855 <sup>a</sup>		4	3.6
Sarsasapogenin			2951	2932		2870 <sup>a</sup>	2862	2854 <sup>a</sup>		5	3.7
Rockogenin		2973 <sup>a</sup>	2953	2929		2874	2861	2851 <sup>a</sup>		6	4.2
<b>20<math>\alpha</math> <math>\Delta^5</math> 3-deoxysapogenins</b>											
Diosgenin	3033		2952	2929	2907	2873	2861	2847	2829 <sup>a</sup>	7	4.1
Yamogenin	3032 3019	2964 <sup>a</sup>	2953	2933	2909 <sup>a</sup>	2874	2864	2849	2829 <sup>a</sup>	8	4.0
<b>20<math>\alpha</math> sapogenin acetates</b>											
Tigogenin		2975 <sup>a</sup>	2952	2930	2909 <sup>a</sup>	2873	2861	2846		9	4.6
Gitogenin		2972 <sup>a</sup>	2952	2929	2909 <sup>a</sup>	2873	2862	2848		10	5.2
Hecogenin		2979 <sup>a</sup>	2953	2930	2908 <sup>a</sup>	2874	2861			11	10
Manogenin			2954	2931	2909 <sup>a</sup>	2874	2863	2854 <sup>a</sup>		12	10
Chlorogenin		2975 <sup>a</sup>	2954	2930	2909 <sup>a</sup>	2874	2861	2851 <sup>a</sup>		13	10
Smilagenin		2972 <sup>a</sup>	2954	2930	2909 <sup>a</sup>	2873	2863	2848		14	4.1
Sarsasapogenin			2953	2935	2910 <sup>a</sup>	2873	2864	2848 <sup>a</sup>		15	4.9
Markogenin			2954	2936	2909 <sup>a</sup>	2875	2866	2852 <sup>a</sup>	2839 <sup>a</sup>	16	10
<b>20<math>\alpha</math><math>\Delta^5</math> sapogenin acetates</b>											
Diosgenin	3034		2953	2931	2908	2873	2861 <sup>a</sup>	2851	2829	17	10
Yuccagenin	3034	2972 <sup>a</sup>	2953	2929	2907	2873	2861 <sup>a</sup>	2848	2830	18	10
Gentrogenin	3034		2955	2930	2908	2873	2860		2832	19	7.5
Kammogenin	3038		2957	2931	2907	2875	2863	2847 <sup>a</sup>	2833	20	10
Yamogenin	3032	2962 <sup>a</sup>	2951	2936 <sup>a</sup>	2909	2874		2852	2831	21	7.5
Correllogenin	3033		2958	2937	2909	2873	2863 <sup>a</sup>	2853 <sup>a</sup>	2831	22	4.0
<b>20<math>\beta</math> sapogenin acetates</b>											
Tigogenin		2992 <sup>a</sup>	2953	2932	2920 <sup>a</sup> 2908 <sup>a</sup>	2872	2860	2851		23	4.2
Gitogenin		2992 <sup>a</sup>	2954	2932	2921 <sup>a</sup> 2907 <sup>a</sup>	2872	2863	2848		24	10
Hecogenin			2953	2932	2907 <sup>a</sup>	2872	2861	2851 <sup>a</sup>		25	10
Manogenin			2953	2932	2907 <sup>a</sup>	2872	2863	2852		26	10
Chlorogenin			2953	2934	2919	2872	2859			27	10
Sarsasapogenin		3002 <sup>a</sup>	2953	2933	2909 <sup>a</sup>	2874	2865			28	4.0
Smilagenin		2993 <sup>a</sup>	2953	2934	2909 <sup>a</sup>	2873	2864			29	4.2
Markogenin		3005 <sup>a</sup>	2954	2931	2911 <sup>a</sup>	2874	2865			30	10
<b>20<math>\beta</math> <math>\Delta^5</math>-sapogenin acetates</b>											
Diosgenin	3033	2992 <sup>a</sup>	2953	2933	2907	2873		2853	2829	31	10
Yamogenin	3032	3008	2954	2936	2908	2873		2854	2831	32	10
<b>20<math>\alpha</math>-dihydrosapogenin acetates (open F ring)</b>											
3-Deoxytigogenin		2962		2927	2903 <sup>a</sup>	2867 <sup>a</sup>	2856	2847 <sup>a</sup>		33	7.5
Tigogenin			2951	2934	2902	2868		2847	2831 <sup>a</sup>	34	7.5
Gitogenin			2953	2934	2900	2864		2847	2834 <sup>a</sup>	35	7.5
<b>20<math>\beta</math>-dihydrosapogenin acetates</b>											
Tigogenin		2990 <sup>a</sup>	2950 <sup>a</sup>	2935	2915	2867 <sup>a</sup>	2849			36	10
Hecogenin		2966 <sup>a</sup>		2935	(2892)		2858			37	10
<b>Cholestane and related compounds (open E and F rings)</b>											
Cholestane			2950 <sup>a</sup>	2929		2867	2856	2848 <sup>a</sup>		38	4.2
Cholestanol			2951 <sup>a</sup>	2933	2907 <sup>a</sup>	2868	2858			39	5.0
Coprostanol			2950	2932	(2885)	2862	2853 <sup>a</sup>			40	5.8

<sup>a</sup> Bands appearing as inflections on more intense bands.

branched-chain or unsaturated hydrocarbons. Pozefsky and Coggeshall (12) observed a 2910-cm.<sup>-1</sup> band for a number of compounds, mostly aldehydes and ketones. It would seem that double bonds and carbonyl groups increase the absorption of nearby methyl

groups around 2910 cm.<sup>-1</sup> A few 20 $\beta$  sapogenin acetates have an additional shoulder at 2920 cm.<sup>-1</sup>, and open F-ring steroids have one at 2900 cm.<sup>-1</sup> The origin of these inflections is unknown.

**2880- to 2830-Cm.<sup>-1</sup> Region.** The

symmetrical vibrations of both CH<sub>3</sub> and CH<sub>2</sub> groups appear in this region, around 2870 and 2860 cm.<sup>-1</sup>, respectively, for the sapogenins examined. The 2870-cm.<sup>-1</sup> CH<sub>3</sub> band is weak in 3-deoxysapogenins compared with the 2860-cm.<sup>-1</sup> CH<sub>2</sub> band. A third band

appears around 2850  $\text{cm}^{-1}$  in tigogenin derivatives and  $\Delta^5$  compounds, but is only an inflection in most other steroidal sapogenins. This band was also assigned to the  $\text{CH}_2$  group because it decreases with increase in ring substitution. The band is almost undetectable in ketonic sapogenins. The 2860- $\text{cm}^{-1}$   $\text{CH}_2$  band almost disappears in  $\Delta^5$  acetates, except for carbonyl compounds. In compounds with open E and F rings, the bands in this region appear at lower frequencies. A band near 2830  $\text{cm}^{-1}$  is associated with  $\Delta^5$  unsaturation, and is stronger in  $\text{C}=\text{O}$  compounds, but no definite assignment can be made.

#### CONCLUSIONS

Structural features of various steroidal sapogenins can be identified from details of their C—H stretching spectra. Of the seven to nine bands observed, five can be associated with specific modes of  $=\text{CH}$ ,  $\text{CH}_2$ , and  $\text{CH}_3$  groups. All observed bands seem to show a strong dependence on the over-all structure of the molecule. Easiest to characterize are 3-deoxysapogenins, sapogenin acetates,  $\Delta^5$  compounds, and open-ring compounds. In 3-deoxysapogenins, the  $\text{CH}_2$  groups absorb so much more than the  $\text{CH}_3$  groups that  $\text{CH}_3$  bands at 2950 and 2870  $\text{cm}^{-1}$  are perceptible only as shoulders on the slopes of strong  $\text{CH}_2$  bands. Acetylation increases the relative intensity of these

$\text{CH}_3$  bands, making them nearly equal to the  $\text{CH}_2$  bands. The presence of two additional bands around 3035 and 2830  $\text{cm}^{-1}$  is sufficient to characterize  $\Delta^5$  unsaturation. In addition,  $\Delta^5$  acetates show increased absorption around 2910  $\text{cm}^{-1}$ , paralleled by decreases near 2930 and 2860  $\text{cm}^{-1}$ . Open-ring compounds have broader and fewer bands, and those below 2880  $\text{cm}^{-1}$  are shifted to lower frequencies. The  $20\beta$  compounds are readily recognized by inflections around 3000  $\text{cm}^{-1}$ , and in some cases also at 2920  $\text{cm}^{-1}$ . The  $\text{CH}_2$  bands of  $20\beta$ ,  $25\text{D}$  acetates are slightly stronger, and the  $\text{CH}_3$  bands weaker than those of the corresponding  $20\alpha$  compounds. *cis*-A/B sapogenins with  $25\text{L}$  methyl groups have a greater absorption area and broader individual bands than their *trans*-A/B,  $25\text{D}$  counterparts. No *trans*-A/B,  $25\text{L}$  compounds and only one *cis*-A/B,  $25\text{D}$  compound were available. The *cis*- and *trans*- $25\text{D}$  compounds were similar, suggesting that the band-broadening and increased area are mainly caused by D, L isomerism in the  $25$  position.

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#### LITERATURE CITED

- (1) Fox, J. J., Martin, A. E., *Proc. Roy. Soc. (London)* **A162**, 419 (1937).
- (2) *Ibid.*, **A167**, 257 (1938).

- (3) *Ibid.*, **A175**, 208 (1940).
- (4) Guertin, D. L., Wiberley, S. E., Bauer, W. H., *J. Am. Oil Chemists' Soc.* **33**, 172 (1956).
- (5) Jones, R. N., Herling, F., *J. Org. Chem.* **19**, 1252 (1954).
- (6) Jones, R. N., Humphries, P., Packard, E., Dobriner, K., *J. Am. Chem. Soc.* **72**, 86 (1950).
- (7) Jones, R. N., Williams, V. Z., Whalen, H. J., Dobriner, K., *Ibid.*, **70**, 2024 (1948).
- (8) Marker, R. E., Wagner, R. B., Ulshafer, P. R., Wittbecker, E. L., Goldsmith, D. P. J., Ruoff, C. H., *Ibid.*, **69**, 2167 (1947).
- (9) Nolin, B., Jones, R. N., *Can. J. Chem.* **34**, 1382 (1956).
- (10) *Ibid.*, p. 1392.
- (11) Nolin, B., Jones, R. N., *J. Am. Chem. Soc.* **75**, 5626 (1953).
- (12) Pozefsky, A., Coggeshall, N. D., *ANAL. CHEM.* **23**, 1611 (1951).
- (13) Saier, E. L., Coggeshall, N. D., *Ibid.*, **20**, 812 (1948).
- (14) Saunders, R. A., Smith, D. C., *J. Appl. Phys.* **20**, 953 (1949).
- (15) Tallent, W. H., Siewers, I. J., *ANAL. CHEM.* **28**, 953 (1956).
- (16) Wall, M. E., *Experientia* **11**, 340 (1955).
- (17) Wall, M. E., Krider, M. M., Rothman, E. S., Eddy, C. R., *J. Biol. Chem.* **198**, 533 (1952).
- (18) Wall, M. E., Serota, S., *J. Am. Chem. Soc.* **78**, 1747 (1956).
- (19) Wall, M. E., Walens, H. A., *Ibid.*, **77**, 5661 (1955).
- (20) *Ibid.*, **80**, 1984 (1958).

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