312. *The Determination of* C-*Methyl Groups in Some Branched-chain Fatty Acids.*

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By using compounds of known constitution it has been found that the Kuhn–Roth method (Ginger's modification) for the estimation of *C*-methyl groups gives one mole of acetic acid (62—99% yield) for each $-CMe_3$, $>CMe_2$, and $\geqslant$CMe group. The method therefore cannot be used as a means of determining for certain the number of methyl groups present in the molecule or their particular nature.

THE Kuhn–Roth procedure for the determination of *C*-methyl groups (Kuhn and Roth, *Ber.*, 1933, **66**, 1274) has been accepted by various workers since it was introduced in 1933. It involves oxidation of the compound by chromic acid to give, theoretically, one mole of acetic acid for every *C*-methyl group per mole of compound.

Kuhn and Roth describe the determination of *C*-methyl groups in a number of complex materials some of which contain *gem*-dimethyl groups. Examination of this original work, however, indicates that the method was tested on complex compounds containing both *gem*-dimethyl and $\geqslant$CMe groups. Lycopene, for example, which contains six $\geqslant$CMe groups as well as two $>CMe_2$ groups gives acetic acid for six *C*-methyl groups in 91% yield or for eight in 69% yield. As the yield of acetic acid varies considerably and may be as low as 62%, analysis of such compounds will not show whether the acetic acid comes from the $\geqslant$CMe groups alone or from both $\geqslant$CMe and $>CMe_2$ groups.

The exact behaviour of the $>CMe_2$ and $-CMe_3$ groups in particular requires elucidation because Milton and Waters (" Methods of Quantitative Microanalysis," E. Arnold & Co., 1949, p. 111) state that $>CMe_2$ and $-CMe_3$ groups yield acetone. The purpose of the work now reported has been to determine the reaction of *gem*-dimethyl and *tert.*-butyl groups and certain other types of side chains in compounds submitted to the Kuhn–Roth procedure as modified by Ginger (*J. Biol. Chem.*, 1944, **156**, 452). Our results are tabulated.

Substance	AcOH, moles per mole of compound	
1, Decanoic acid	0·99	1 Me in 99% yield
2, Stearic acid	0·96	1 ,, 96% ,,
3, *iso*Valeric acid	0·78	1 ,, 78% ,,
	* 0·84	1 ,, 84% ,,
4, 2-*n*-Butylhexanoic acid †	1·64	2 ,, 82% ,,
5, Pivalic acid	0·72	1 ,, 72% ,,
6, 2 : 2-Dimethyloctanoic acid †	1·64	2 ,, 82% ,,
7, α-Methyl-α-*n*-propylvaleric acid	2·10	3 ,, 70% ,,
8, α-Ethyl-α-*n*-propylvaleric acid	1·86	3 ,, 62% ,,
9, αα-Diethylvaleric acid	1·86	3 ,, 62% ,,
10, 4 : 4-Dimethyltetradecanoic acid †	1·70	2 ,, 85% ,,
	1·90	2 ,, 95% ,,
11, 3-Methylbutan-2-one	* 1·62	2 ,, 81% ,,
	1·68	2 ,, 84% ,,
12, Mesityl oxide	* 1·54	2 ,, 77% ,,
13, Acetone	* 0·74	1 ,, 74% ,,

* Acetic acid identified as its *p*-bromophenacyl ester. † Geneva convention, $CO_2H = 1$.

The relatively high yield of acetic acid found on oxidation of $>CMe_2$ groups (compounds **3**, **6**, **10**, **11**, **12**) and $-CMe_3$ groups (compound **5**) precludes the formation of acetone under the conditions used. Acetone itself underwent oxidation to acetic acid in 74% yield even although there is the possibility of loss owing to volatility. It was thought that mesityl oxide, owing to its structure, would oxidise readily to give acetone but acetic acid was obtained for the $>CMe_2$ and $\geqslant$CMe groups in 77% yield. It is thus clear that $>CMe_2$ and $-CMe_3$ groups give acetic acid and not acetone.

Duplicate analyses (see table) were fairly reproducible. However, Cason and Sumrell (*ibid.*, 1951, **192**, 411) state that their microanalyst " was unable to obtain satisfactorily consistent results by the method of Ginger." By digesting the fatty acids at 120° in a sealed tube for 11—14 hours reasonably consistent results (75—90% yield) were obtained on synthetic branched-chain acids but even with this modification it is reported that some analyses gave very low results. In the present work, with digestions at 100°, the only two compounds to give less than a 70% yield of acetic acid per group were two acids of similar structure each containing three $\geqslant$CMe groups.

No conclusive evidence of the amount of branching in a chain can be obtained by the Kuhn–Roth method, as the results show that the acid could come from a $\geqslant$CMe, $>CMe_2$, or $-CMe_3$ group. Also, as the yield of acetic acid may be as low as 60% in branched-chain compounds, if more than three *C*-methyl groups are present then the results cannot be interpreted with any degree of certainty.

Experimental.—*C*-Methyl determinations on 10—20-mg. samples of the compounds listed were carried out in the micro-acetyl apparatus described by Pregl (transl. Grant, " Quantitative Organic Microanalysis," J. & A. Churchill Ltd., 1945, p. 162). Ginger's modification was used except that all samples were digested for 1½—2 hours by immersing the reaction flask deeply in a boiling-water bath. In cases indicated in the table the acetic acid in the distillate was identified as the *p*-bromophenacyl ester.

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