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STUDIES IN SESQUITERPENES—XXVII*

DIFFERENTIAL KUHN–ROTH C-METHYL ESTIMATION†‡

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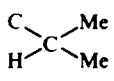
Abstract—A method, based on Kuhn–Roth C-Me estimation at two different temperatures, is described, which yields useful information about the number and nature of quaternary methyls in isoprenoids, especially sesquiterpenes.

AN important item of information sought for the structure determination of organic natural products, especially isoprenoids, is the number and nature of C-Me groups. C-Me determination by Kuhn–Roth chromic acid oxidation method[1] proved of considerable consequence. Later, IR spectrophotometry provided a superior method, when suitable reference compounds were available.[2–6] In recent years, this information is most conveniently and effectively provided by PMR spectroscopy. However, often situations arise when further refinement of the information becomes valuable. Thus, for example, it is desirable to establish whether the two Me groups showing up in a PMR spectrum as sharp signals are present as I, II or III. Though, PMR spectrometry can distinguish between situations I and II, often measurements



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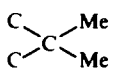

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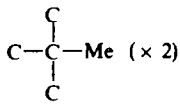
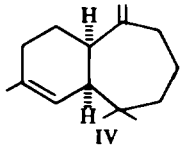


III

at a different frequency will be required. On the other hand, a distinction between II and III, in an unknown, cannot be made by the existing methods.**

During our work on the structure determination of himachalenes (IV, V)[8] and isolongifolene (VI),[9] we were faced with similar situations and, we now report on a method, based on Kuhn–Roth C-Me estimation at two different temperatures, which provides some answer in the field of sesquiterpenoids.



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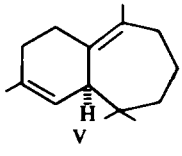

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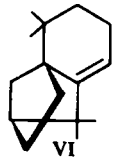

VI

* Part XXVI: *Tetrahedron* **22**, 2319 (1966).

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‡ Abstracted from the Ph.D thesis (Bombay University, 1966) of V. S. Pansare.

** In principle, this distinction is possible by IR spectrometry and the method has been used in certain cases.[7] However, the results are often vitiated due to the presence of other Me groups and due to the general variation in the ε value of the Me group absorption.

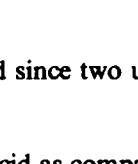
From a survey of the methods and results of Kuhn–Roth C-Me estimation,[1b] it becomes clear that a *gem*-dimethyl residue (II) can at best contribute a maximum of one mole equivalent of acetic acid and that, dimethylsuccinic and dimethylmalonic acids, to which many compounds containing the unit II would be ultimately broken down by the chromic acid reagent, are highly resistant[10] to further attack under the usual conditions. It is the inertness of these acids to further oxidation which is responsible for the low acetic acid values in the Kuhn–Roth estimations on compounds with the structural feature II. In order to determine the conditions for the further breakdown of αα-dimethylsuccinic acid, its oxidation with chromic acid under various conditions (Table 1) was investigated. Since it has been reported[11] that significant losses of acetic acid occur at higher temperatures, the extent of loss was ascertained experimentally (Table 2). From these data it is clear that αα-dimethylsuccinic acid gives negligible amounts of acetic acid under the usual conditions[1a] of Kuhn–Roth estimation (120°; 1·5 hr), but appreciable quantities of acetic acid are produced when the oxidation is carried out at 140° (10 hr). This value will appreciate significantly,

TABLE 1. OXIDATION OF αα-DIMETHYLSUCCINIC ACID

Temp	Time (hr)	Yield of acetic acid (mole equiv)
120°	1·5	0·09
125°	1·5	0·12
130°	1·5	0·34
125°	2·5	0·14
140°	10	0·54

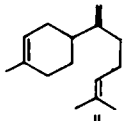
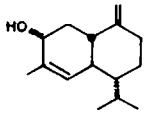
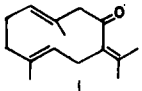
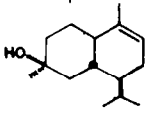
TALBE 2. OXIDATION OF ACETIC ACID

Temp	Time (hr)	Loss of acetic acid (%)
140°	8	9·5
140°	16	20·6
140°	24	30·9

when the loss of acetic acid by further oxidation is also taken into account. Furthermore, since under the usual conditions of Kuhn–Roth estimation (120°, 1·5 hr), compounds with quaternary Me groups (III) also give poor yields of acetic acid,[1] these compounds also may be expected to furnish extra acetic acid under the more drastic conditions (140°, 10 hr). Thus, it was argued that the difference in the amount of acetic acid produced under the two conditions, may possibly provide a measure of the number of quaternary Me's and since two units of type  should give almost twice the amount of acetic acid as compared to that from II, a differentiation between II and III should become feasible.

To test the above reasonings, Kuhn–Roth estimations of a number of sesquiterpenes of known structures have been carried out under the two conditions. Table 3 shows the results obtained with sesquiterpenes containing no quaternary Me's. As expected ΔAcOH is zero or negative, thus conforming to our expectations that in the absence of

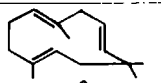
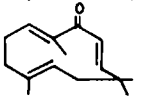
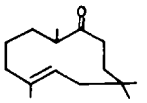
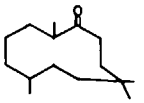
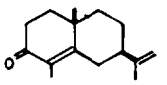
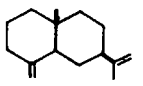
TABLE 3. DIFFERENTIAL KUHN–ROTH ESTIMATIONS ON SESQUITERPENES WITH NO QUATERNARY METHYLS

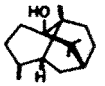
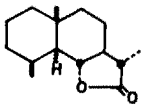
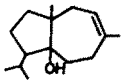
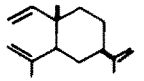
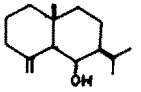
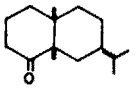
No.	Compound	Structure	Yield of AcOH in mole equiv		ΔAcOH* (mole)
			120°/1·5 hr	140°/10 hr	
1	β-Bisabolene		2·33	2·22	−0·11
2	Khusinol		1·88 1·86	1·72 1·79	−0·16 −0·07
3	Germacrone		2·28 2·24	2·29 2·24	+0·01 0·00
4	δ-Cadinol		2·21 2·20	2·13 2·23	−0·08 +0·03

* ΔAcOH = yield of AcOH at high temp minus the yield of AcOH at lower temp.

quaternary Me groups, the usual conditions suffice for complete oxidation to acetic acid. Table 4 summarizes the results with a number of sesquiterpenes having at least one quaternary Me. As can be seen ΔAcOH is always positive, the average ΔAcOH value per quaternary Me group being 0·42 mole. By taking this value as equivalent to one quaternary Me, the number of such Me's has been calculated and the results compared with the actual values and, it may be noted that except in the case of compounds No. 6, 7 and 10, the results are fairly good and in conjunction with the results given in Table 3, have considerable diagnostic value. Thus almost nil ΔAcOH indicates the absence of any quaternary Me's and, if necessary, can be used to differentiate between I and II (or III). Furthermore, since a structural residue of the type II is equivalent to one quaternary Me in the Kuhn–Roth estimation, the method can be used to decide whether a compound of unknown structure showing two quaternary Me signals in its PMR spectrum, has these present as a *gem*-dimethyl group (II) or two separate quaternary Me's (III).

Table 4. Differential Kuhn–Roth estimations on sesquiterpenes with quaternary methyls

No.	Compound	Structure	Yield of AcOH in mole equiv*		ΔAcOH† (mole)	No. of quaternary Me equiv‡	
			120°/1·5 hr	140°/10 hr		Found	Actual
1	Humulene		1·51	2·01	+0·50	1·2	1
2	Zerumbone		1·75	2·15	+0·40	1·0	1
3	Tetrahydrozerumbone		1·64	2·13	+0·49	1·2	1
4	Hexahydrozerumbone		1·57	1·91	+0·34	0·8	1
5	Longifolene		0·60	1·47	+0·87	2·1	2
6	α-Cyperone		2·03	2·22	+0·19	0·5	1
7	β-Selinene		1·51	1·73	+0·22	0·5	1

8	Patchouli alcohol		1·32	1·73	+0·41	1·0	1
9	Santanolide–C		1·55	1·97	+0·42	1·0	1
10	Carotol		1·91	2·10	+0·19	0·5	1
11	β-Elemene		1·44	1·96	+0·52	1·2	1
12	Junenol		1·13	1·54	+0·41	1·0	1
13	Valeranone		1·00	1·79	+0·79	1·9	2
14	Cedrol		0·91	1·50	+0·59	1·4	1

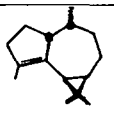
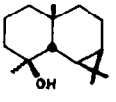
* Mean of two values.

† See footnote to Table 3.

‡ No. of quaternary Me equiv = ΔAcOH/average ΔAcOH (i.e. 0·42); two Me's in type II are at best equiv to one AcOH in Kuhn–Roth estimations and hence such groups are counted as one quaternary Me equiv.

If the grouping II or III constitutes a part of a cyclopropane derivative, the compound may behave either as belonging to the class of compounds without quaternary Me's or as belonging to those with quaternary Me's, depending on the structure(s) of the product(s) resulting from cleavage of the cyclopropane ring by the H_2SO_4 present in the reaction mixture. Table 5 gives the results obtained with two such compounds and as can be expected from their structures the *gem*-dimethyl group present on the cyclopropane ring is not expected to contribute to the acetic acid any additional amount at higher temp as indeed has been found to be the case.

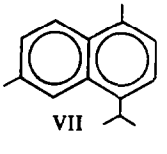
TABLE 5. DIFFERENTIAL KUHN–ROTH ESTIMATIONS ON SESQUITERPENES WITH CYCLOPROPANE RINGS*

No.	Compound	Structure	Yield of AcOH in mole equiv		ΔAcOH (mole)	No. of quaternary Me equiv (Found)
			120°/1·5 hr	140°/10 hr		
1	α-Gurjunene		2·40	2·22	−0·18	Nil
2	Maaliol		1·72	2·05	+0·33	0·8

* See footnotes to Table 4.

As mentioned earlier, the need for a method which could differentiate between the possibilities II and III, arose in connection with the structure determination of sesquiterpenes himachalenes[9] and isolongifolene.[10] We discuss below the data obtained with these compounds which helped in giving the necessary information.

The himachalenes are the sesquiterpenes present in the essential oil of *Cedrus deodara* Loud. These compounds on dehydrogenation with selenium gave cadalene (VII) and 2-methyl-6-(*p*-tolyl)-heptane (VIII) as the major products. However, from



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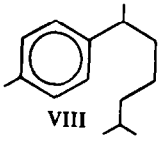

VIII

various other considerations it was concluded that himachalenes do not contain an isopropyl group and in all probability these two Me's are present as II. This was clearly supported by the differential Kuhn–Roth estimations (Table 6) on both α- and β-himachalenes, the structures of which are now well-established as IV and V respectively. Likewise, in isolongifolene it was desired to establish, whether its four quaternary Me's (PMR) are present as II and III or as two units of II. Differential Kuhn–Roth estimations (Table 6) clearly showed that two units of II must be present, which is fully consistent with the structure VI for isolongifolene, now well-secured by synthesis.[12]

TABLE 6. DIFFERENTIAL KUHN–ROTH ESTIMATIONS ON HIMACHALENES AND ISOLONGIFOLENE*

No.	Compound	Yield of AcOH in mole equiv		ΔAcOH (mole)	No. of quaternary Me equiv (Found).
		120°/1·5 hr	140°/10 hr		
1	α-Himachalene	1·09	1·56	+0·47	1·1
2	β-Himachalene	1·58	2·02	+0·44	1·0
3	Isolongifolene	0·76	1·58	+0·82	1·9

* see footnotes to Table 4.

EXPERIMENTAL

Sesquiterpene samples. All samples used were GLC pure and were available in our laboratory.

Reagents. Oxidation mixture was prepared by dissolving 16·70 g CrO_3 (B.D.H., AR) in 100 ml distilled water and, adding 25 ml conc H_2SO_4 (AR, sp. gr. 1·84) to it with cooling. $BaCl_2$ used was B.D.H. A.R.

Apparatus. For oxidation thick-walled Pyrex tubes of following dimensions were used: internal diam, 10 mm; external diam, 12 mm; length, 35 cm.

Heating was carried out in a micro Carius furnace[13] with a heating block fabricated to accommodate four tubes, 35 cm in length. The heating was controlled by an electronic relay within ±2°.

Procedure. About 5–7 mgm of the compound was weighed out accurately in a micro carius glass tube. After chilling the tube in ice cold water, 5 ml of the oxidation mixture was added to the sample. The tube was then sealed at a length of 30 cm and after the carius furnace attained the desired temp, it was introduced cautiously into one of the pockets of the furnace and the lid properly closed. When the solubility of the compound in the oxidation mixture was found to be somewhat unsatisfactory, the tube was taken out occasionally and turned carefully several times to establish a better contact of the sample with the oxidation mixture. After heating the tube for the desired length of time at the required temp it was taken out carefully and placed in a beaker containing water. After chilling the tube with ice-cold water to avoid the loss of AcOH, the tube was opened as usual and the mixture transferred to the usual distillation assembly.[14] To ensure a quantitative transfer of the mixture, the tube was washed thrice with 1 ml portions of distilled water and the washings transferred to the distillation assembly. The distillation of AcOH was then carried out without neutralizing the excess chromic acid. After ascertaining the absence of H_2SO_4 in the distillate with a small quantity of $BaCl_2$ the distillate was titrated against 0·01N NaOH to end point and the results calculated in the usual manner.

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