

## Synthesis of monoketo and monohydroxy eicosanoic acids and esters with substituents at odd-numbered (3-13) carbons

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In this study, monoketo and monohydroxy eicosanoic acids and their methyl esters with the position of the substituent on odd numbered carbon atoms from 3 to 13 were synthesized with high purity. Furthermore, the semicarbazone and anilide derivatives of the obtained keto acids were prepared. They were characterized by TLC, IR and <sup>1</sup>H-NMR spectroscopy and their physical and chemical properties were established.

*Keywords:* monoketo, monohydroxy, eicosanoic acid methyl ester.

### INTRODUCTION

Several keto and hydroxy alkanolic acids have been isolated from natural sources. The low molecular weight keto alkanolic acids are important biological substances which are used as intermediates in organic syntheses. The hydroxy acids occur more frequently in nature as constituents of natural lipids, various natural waxes (caranday, carnauba, licuri, conifer, jalop, tree-bark waxes, wool wax and beeswax), brain lipids (cerebrosides) and some seed fats.<sup>1</sup>

Among the synthesized keto and hydroxy eicosanoic acids; 5- and 11-oxoeicosanoic acids have been detected in cheese by HPLC,<sup>2</sup> 9-, 11- and 15- keto isomers as their methyl esters in milk fat by GC-Mass spectroscopy,<sup>3</sup> 3-hydroxyeicosanoic acid was found in the microorganism *Legionella pneumophila*<sup>4</sup> and *Mycobacterium tuberculosis*<sup>5</sup> and in the leaves and roots of the plant *Centaurea aspera*,<sup>6</sup> 8-, 9-, 11-, 12- and 15-hydroxyeicosanoic acids were determined in lipids and 13-hydroxy in *Apis mellifera* beeswax by GC-Mass spectroscopy.<sup>7,8</sup>

The hydroxy fatty acids<sup>9–12</sup> mentioned in the literature are valuable starting compounds used in the preparation of numerous textile auxiliaries, detergent, dispersion and emulsion reagents<sup>13</sup> and play an important role in cancer chemotherapy.<sup>14,15</sup> Furthermore, hydroxy acids are used as geochemical indicators.<sup>16</sup>

3-Oxoeicosanoic acid was prepared according to the acetoacetic ester method.<sup>17</sup> 5-Oxoeicosanoic acid was synthesized by the reaction of malonic ester.<sup>18</sup> The 9-,<sup>19,20</sup>

11-<sup>21</sup> and 13-oxo<sup>21</sup> isomers were obtained *via* the Blaise condensation method and 3-hydroxyeicosanoic acid by the reduction of its keto ester with Raney Ni.<sup>22</sup>

In this work, twelve odd numbered monoketo and monohydroxy eicosanoic acids and their methyl esters with the substituent position from 3 to 13 were synthesized with high purity. Their semicarbazone and anilide derivatives were also prepared.

The 5-, 7-, 9-, 11-, 13-monohydroxy eicosanoic acids and their methyl esters were obtained for the first time in this work. 7-Oxoeicosanoic acid, its methyl ester and all the mentioned semicarbazone and anilide derivatives, except the 9-semicarbazone eicosanoic acid, are novel.

The hydroxy esters were synthesized by the reduction of their corresponding keto esters. The reductions were accomplished by the use of NaBH<sub>4</sub>.<sup>23,24</sup> The keto esters were prepared according to the acetoacetic ester and Blaise condensation methods.<sup>25</sup> All the esters were hydrolyzed to their corresponding acids. The physical and chemical properties of the synthesized compounds were established and their purity was checked by TLC, as well as IR and <sup>1</sup>H-NMR spectroscopy.

The physical, chemical and spectral data summarized in this work is expected to fill the void in previous publications and to be helpful for the identification of similar compounds found in nature.

#### RESULTS AND DISCUSSION

7-Oxoeicosanoic acid and its methyl ester, 5-, 7-, 9-, 11-, 13-hydroxyeicosanoic acids and their methyl esters are new compounds. 3-Oxoeicosanoic acid methyl ester was synthesized by the reaction of acetoacetic ester with steroyl chloride, by the acetoacetic ester method. The yield was 70 %. The other keto esters were synthesized by the Blaise condensation reaction<sup>25</sup> with typical yields of 45 %.

The hydroxy esters mentioned in this study were obtained by the reduction of their keto esters by NaBH<sub>4</sub>.<sup>23,24</sup> with typical yields of 85 %. The obtained keto and hydroxy esters were hydrolyzed to their corresponding acids. The keto and hydroxy esters and acids synthesized were purified by crystallization and their degree of purification was monitored by thin layer chromatography.

TABLE I. Melting points of the studied monoketo eicosanoic acids and their methyl esters

Keto acids			Keto esters	
M.p. (°C)	M.p. (°C) lit.	Keto position	M.p. (°C)	M.p. (°C) lit.
97–98	100.5–101 <sup>17</sup>	3	51.5–53	55–56 <sup>17</sup>
94–95	93 <sup>18</sup>	5	59.5–60.5	–
87–88	–	7*	54.5–55.5	–
81–82	78–80 <sup>19</sup> ; 86.2–87.6 <sup>20</sup>	9	53.5–54.5	55.2–56.5 <sup>20</sup>
86–87	86.5–87.3 <sup>21</sup>	11	54–55	54.5–55.5 <sup>21</sup>
111–112	87.5–87.8 <sup>21</sup>	13	55–55.5	55–55.5 <sup>21</sup>

(\*) 7-Oxoeicosanoic acid methyl ester was synthesized for the first time in this work. Calculated C % 74.06, H % 11.84; Found C % 73.60, H % 11.97.

TABLE II. Melting points and microanalysis of monohydroxy eicosanoic acids and their methyl esters

Hydroxy acids		Hydroxy ester			
M.p. (°C)	M.p. (°C) lit.	Calculated: C % 73.12, H % 12.27		Hydroxy position	M.p. (°C)
		Found			
		C %	H%		
91.92.5	93.2–93.4 <sup>22</sup>	–	–	3	56.5–57.5
86–87.5	–	72.81	12.01	5	57–57.5
83.5–84	–	72.87	11.89	7	57.5–58
80–81	–	73.57	12.78	9	53.5–55
80.5–82	–	73.60	12.14	11	53–54.5
110–112	–	73.63	12.60	13	52.5–53.5

Melting points and microanalysis of the studied monoketo and monohydroxy acids and their methyl esters are summarized in Tables I and II. There is no discernible correlation between the melting point and the keto position.

The semicarbazone and anilide derivatives of these keto acids were obtained. Only the 9-semicarbazone eicosanoic acid has been synthesized, previously. The other five semicarbazones and six anilides synthesized in this work are new compounds. The melting points and microanalysis are given in Table III.

TABLE III. Melting points and microanalysis of the synthesized anilide and semicarbazone derivatives of the ketoeicosanoic acids

Semicarbazone derivatives						Anilide derivatives			
Calculated (%): C 65.76, H 10.77, N 10.96						Calculated (%): C 77.70, H 10.79, N 3.49			
Found (%)			M.p. (°C)	M.p. (°C) lit.	Keto position	M.p. (°C)	Found (%)		
C	H	N					C	H	N
66.03	10.95	11.12	124–124.5	–	3	80–82	77.84	10.64	3.02
66.25	11.16	10.57	116–116.5	–	5	98.5–100.5	77.96	10.86	3.43
65.91	11.02	11.16	100–101.5	–	7	99.5–100	77.31	11.17	3.26
–	–	–	119–119.5	110–112 <sup>19</sup>	9	98.5–99.5	77.45	10.93	3.31
66.17	11.19	11.23	121–122	–	11	100–101	77.63	10.99	3.71
66.13	10.87	11.05	109–110	–	13	101–102.5	77.51	11.23	3.65

The IR and <sup>1</sup>H-NMR spectroscopy results of synthesized compounds are summarized in Table IV.

TABLE IV. The IR and  $^1\text{H-NMR}$  spectra of the ketoeicosanoic acids methyl esters, hydroxy eicosanoic acids, anilide and semicarbazone derivatives of the keto eicosanoic acids

Compound	IR ( $\text{cm}^{-1}$ ) : as KBr pellets	$^1\text{H-NMR}(\text{CDCl}_3)$ : $\delta$ (ppm)
3-,5-,7-,9-,11-,13-Monoketo eicosanoic acid methyl ester isomers	(1715–1705) (ketone C=O), (1738–1754) (ester C=O), (1169–1200) (C-O)	–
7-Oxoeicosanoic acid methylester	1708 (ketone C=O), 1746 (ester C=O), 1184 (C-O)	0.88 ( <i>t</i> , $-\text{CH}_3$ ), 1.25 ( <i>m</i> , $-(\text{CH}_2)_{11}-$ ), 1.59 ( <i>m</i> , $(\text{CH}_2)_3-$ ), 2.38 ( <i>m</i> , $-\text{CH}_2\text{COCH}_2-$ ), $-\text{CH}_2\text{COOCH}_3$ ), 3.66 ( <i>s</i> , $-\text{OCH}_3$ )
3-,5-,7-,9-,11-,13-Menohydroxy eicosanoic acid isomers	(3100–3540) (O–H), (1702–1715) (C=O), (1230–1300) (C–O)	–
7-Hydroxyeicosanoic acid*	3392 (O–H), 1715 (C=O), 1292 (C–O)	0.5 ( <i>t</i> , $-\text{CH}_3$ ), 0.7 ( <i>m</i> , $-(\text{CH}_2)_{11}-$ ), 1.2 ( <i>m</i> , $-\text{CH}_2\text{CH}_2(\text{CH}_2)_2\text{COOH}$ ), 1.38 ( <i>m</i> , $-\text{CH}_2\text{CHOHCH}_2-$ ), 1.8 ( <i>m</i> , $-\text{COOH}$ , $-\text{OH}$ ), 2.5 ( <i>m</i> , $-\text{CH}_2\text{CH}_2\text{COOH}$ ), 3.6 ( <i>m</i> , $-\text{CHOH}$ )
3-,5-,7-,9-,11-,13-Monoketo eicosanoic acid semicarbazones	(3461–3400) (O–H), (3265–3215) ( $-\text{NH}_2$ ), (1601–1580) (N–H), (1711–1708) (C=O), (1670–1597) ( $=\text{N}$ , $\text{NHCO}$ )	–
5-Oxoeicosanoic acid semicarbazone	3461 (O–H), 3254 ( $-\text{NH}_2$ ), 1585 (N–H), 1708 (C=O), 1661 ( $=\text{N}$ , $\text{NHCO}$ )	0.88 ( <i>t</i> , $-\text{CH}_3$ ), 1.25 ( <i>m</i> , $-(\text{CH}_2)_{13}-$ ), 1.77 ( <i>m</i> , $-\text{CH}_2\text{CH}_2\text{COOH}$ ), 1.91 ( <i>t</i> , $-\text{CH}_2\text{COOH}$ ), 2.25 ( <i>m</i> , $-\text{CH}_2\text{CNCH}_2-$ ), 6.1 ( <i>s</i> , $-\text{NH}_2$ ), 9.65 ( <i>s</i> , $-\text{COOH}$ )
3-,5-,7-,9-,11-,13-Monoketo eicosanoic acid anilide isomers	(3354–3330) (1586–1560) (N–H), (1682–1672) (N–CO), (1621–1617) (ArC–C), (1730, 1725) (C=O)	–
5-Oxoeicosanoic acid anilide	3334, 1564 (N–H), 1673 (N–CO), 1619 (ArC–C), 1727 (C=O)	0.88 ( <i>t</i> , $-\text{CH}_3$ ), 1.25 ( <i>m</i> , $-(\text{CH}_2)_{12}-$ ), 1.56 ( <i>s</i> , $-(\text{CH}_2)_{12}\text{CH}_2\text{CH}_2-$ ), 1.99 ( <i>t/t</i> , $-\text{CH}_2\text{CH}_2\text{CONH}-$ ), 2.39 ( <i>t/t</i> , $-\text{CH}_2\text{COCH}_2-$ ), 2.56 ( <i>t</i> , $-\text{CH}_2\text{CONH}-$ ), 7.1 ( <i>t</i> , $-\text{NH}-$ ) 7.3 ( <i>m</i> , $-\text{C}_6\text{H}_5-$ )

## EXPERIMENTAL

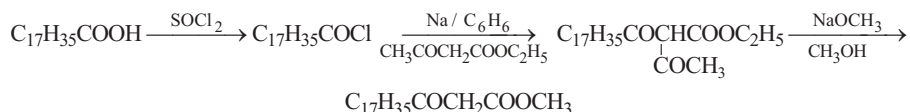
*General considerations*

The spectral data were obtained with the aid of the following instruments; IR: Perkin-Elmer, Model 933 (as 1% KBr pellets).  $^1\text{H-NMR}$ : Varian EM 390 Model, 90 MHz, the chemical shifts are given in ppm relative to the internal standard TMS ( $\delta = 0$  ppm). The melting points were determined with a Buchi Model SMP-20 melting point apparatus and reported without correction. The purity of the individual isomers were checked by TLC with plates covered with Kieselgel 60 HF<sub>254</sub> of 0.2 mm thickness. The plates were activated before

use by heating at 110 °C for 30 min. The samples were applied as dilute (*ca.* 1 %) solutions in chloroform. The plates were developed in tanks lined with solvent soaked filter paper using developing solvents A and B for esters and acids, respectively. A = diethyl ether : petroleum ether = 1 : 1 (v/v) B = chloroform : methyl alcohol : acetic acid = 100 : 10 : 1 (v/v/v). The spots were visualized by spraying 25 % H<sub>2</sub>SO<sub>4</sub> and charring at 200 °C or by detection with anisaldehyde. The reagents used were obtained from Merck.

### 3-Oxoicicosanoic acid methyl ester

This compound was prepared according to the acetoacetic ester reaction.<sup>17</sup> A mixture of 8.5 g of ethyl acetoacetate, 1.25 g of sodium and 70 ml of absolute benzene was refluxed for 2 h with stirring. It was cooled and then 0.05 mol of stearoyl chloride was added under stirring. It was refluxed for another 15 min, cooled and acidified. The upper benzene phase was separated by adding 10 % ethanol and dried over Na<sub>2</sub>SO<sub>4</sub>. The benzene was removed by distillation and a mixture of 45 ml of absolute methanol and 1.5 g of metallic sodium were added to the residue. The mixture was left at room temperature (20–22 °C) for 8 h, then acidified, extracted with ether, washed, dried and evaporated. The reaction was monitored by TLC in solvent A and the crude keto ester was recrystallized from petroleum ether (40–60 °C). Recrystallization was repeated until the product reached constant melting point.

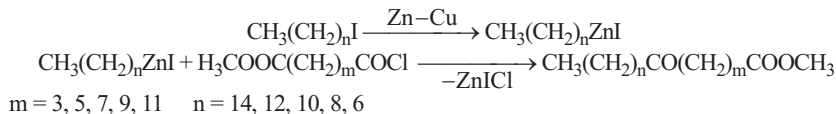


### Stearoyl chloride<sup>26</sup>

Stearic acid (1 mol) was added to a Claisen flask fitted with a reflux condenser in the short neck and a dropping funnel in the long neck. Thionyl chloride (1.2 mol) was added slowly through the dropping funnel over a period of 30–40 min. The flask was then heated gently on a water bath and refluxed for 30 min.  $n_D^{25} = 1.4512$  (lit.  $n_D^{24} = 1.4523$ )  $Kp_{15} = 215$  °C (95 %)

### 5-, 7-, 9-, 11- and 13-Oxoicicosanoic acid methyl esters

These compounds were synthesized by the Blaise condensation reaction.<sup>25</sup> First, a Zn-Cu alloy was prepared using 40 g of zinc and 3 g of copper dust. Using a three-necked flask, 0.05 mol of alkyl iodide and 2.9 g of toluene were added slowly from a dropping funnel into a mixture of 1.4 g of ethyl acetate and 3.5 g of Cu-Zn alloy, under stirring and gentle heating. After the addition of the reagents was completed, the mixture was refluxed for 1–3 h. Then, the flask was cooled down and under stirring, 0.04 mol of the appropriate diacid monoester monochloride was added. After the addition was completed, the mixture was stirred for 1 h. Hydrolysis with 20 ml distilled water and 20 ml 10 % HCl gave an upper ether phase which was separated and washed consecutively with dilute HCl, 10 % Na<sub>2</sub>CO<sub>3</sub> and finally distilled water. After drying over anhydrous Na<sub>2</sub>SO<sub>4</sub>, the solvent was distilled off. The solid product was recrystallized from petroleum ether (40–60 °C). Recrystallization was repeated until the product reached a constant melting point.



### Diacidmonomethyl esters<sup>27</sup>

Into a 500 ml round-bottomed flask provided with a reflux condenser were added 0.3 mol diacid, 15 ml of absolute methanol, 4 ml of concentrated hydrochloric acid. The flask was cautiously heated at first until the mixture becomes homogeneous and then refluxed for 8 h. The mixture was transferred to a Claisen flask with a fractionating side arm and fractionated under reduced pressure. C<sub>5</sub> diacid monomethylester,  $Kp_{20} = 153$  °C,  $n_D^{19} = 1.4352$  (47 %); C<sub>7</sub> diacid monomethylester,  $Kp_{18} = 181$  °C,  $n_D^{29} = 1.436$ , (32 %); C<sub>9</sub> diacid monomethylester,  $Kp_{20} = 200$  °C,  $n_D^{18} = 1.446$ , (41 %); C<sub>11</sub> diacid monomethylester, m.p. = 41–42 °C, (45 %); C<sub>13</sub> diacid monomethylester, m.p. = 47–48.5 °C, (35 %).

### *Diacid monoester monochloride*

The monoester monochlorides were prepared from the diacid monoesters and thionyl chloride as described for stearoyl chloride.

C<sub>5</sub> diacid monomethyl ester–monochloride,  $Kp_{10} = 98\text{ }^{\circ}\text{C}$ ,  $n_D^{30} = 1.4405$ , (92 %); C<sub>7</sub> diacid monomethyl ester monochloride,  $Kp_7 = 112\text{ }^{\circ}\text{C}$ ,  $n_D^{27} = 1.4422$ , (92 %); C<sub>9</sub> diacid monomethyl ester monochloride,  $Kp_{11} = 155\text{ }^{\circ}\text{C}$ ,  $n_D^{18} = 1.45$ , (88 %); C<sub>11</sub> diacid monomethyl ester monochloride,  $Kp_{10} = 165 - 166\text{ }^{\circ}\text{C}$ ,  $n_D^{16} = 1.456$ , (85 %); C<sub>13</sub> diacid monomethyl ester monochloride,  $Kp_{11} = 172\text{ }^{\circ}\text{C}$ ,  $n_D^{27} = 1.459$ , (90 %).

### *3-Oxoecosanoic acid*

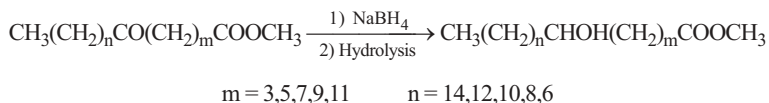
The 3-oxoecosanoic acid methyl ester was hydrolyzed to its acid by acid hydrolysis.<sup>17</sup> The methyl ester (0.5 g) was dissolved in 10 ml glacial acetic acid and approximately 1 to 5 ml concentrated hydrochloric acid added dropwise until the solution became slightly turbid. The mixture was allowed to stand at room temperature for 48 h. The resulting white crystals were collected and dried at room temperature over phosphorus pentoxide. The solid product was recrystallized three times from acetone. The high purity of the β-keto ester is essential for the success of this procedure.

### *5-, 7-, 9-, 11- and 13-Oxoecosanoic acids.*

The appropriate keto esters were hydrolyzed to their acids by base hydrolysis. The keto ester (0.1 mol) was hydrolyzed with 0.5 mol of KOH, 100 ml of methanol and 30 ml of H<sub>2</sub>O. The reaction was monitored by TLC (Solvent B). The acid was crystallized from acetone or petroleum ether (60–80 °C).

### *3-, 5-, 7-, 9-, 11- and 13-Hydroxyecosanoic acids methyl esters*

All the keto esters were reduced with NaBH<sub>4</sub><sup>23,24</sup> to obtain the hydroxy esters. Sodium borohydride (0.005 mol), THF (25 ml) and methanol (25 ml) were mixed at room temperature and 0.005 mol of the keto ester was added. The end of the reaction was monitored by TLC (Solvent A). Then the mixture was acidified with 10 % HCl and ether was added. The ether phase was washed with 10 % Na<sub>2</sub>CO<sub>3</sub> followed by distilled water. After drying over anhydrous Na<sub>2</sub>SO<sub>4</sub>, the solvent was distilled off. The hydroxy esters obtained were hydrolyzed to their acids by base hydrolysis.



### *Preparation of the semicarbazone derivatives<sup>28</sup>*

Semicarbazide hydrochloride (1 g) and sodium acetate (1.5 g) were used for the derivatisation of the keto acid (1 g). The solid products were recrystallized from methanol.

### *Preparation of the anilide derivatives<sup>29</sup>*

For 1 g of keto acid, 2 ml of SOCl<sub>2</sub> and 1 g of aniline were used. The solid products were recrystallized from distilled water or ethanol.

## CONCLUSION

The keto and hydroxy eicosanoic acids can be isolated from natural sources by combining various classical methods such as distillation, chromatography, extraction and crystallization, as well as other special techniques, but the products obtained in this way, usually, contain impurities due to the possible natural side-reactions involving oxidation and polymerization. Therefore, the physical, chemical and spectral data of the newly synthesized acids and esters of high purity will be helpful in identifying these products recovered from natural sources.

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## ИЗВОД

СИНТЕЗА МОНОКЕТО– И МОНОХИДРОКСИ– ЕИКОЗАНСКИХ КИСЕЛИНА И  
ЕСТАРА СА СУПСТИТУЕНТИМА НА НЕПАРНИМ (3–13) УГЉЕНИКОВИМ  
АТОМИМА

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Синтетизоване су монокето- и монохидрокси- еикозанске киселине и њихови метил-естри високе чистоће са супституентима на непарним угљеничним атомима од 3 до 13. Након тога, синтетизовани су семикарбазонски и анилидни деривати добивених кето-киселина. Карактеризација је извршена помоћу TLC, IR и <sup>1</sup>H-NMR спектроскопије, а утврђене су и њихове физичке и хемијске особине.

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## REFERENCES

1. Markley, *Fatty Acids* **1** (1947) 69
2. E. Y. Brechany, W. W. Christie, *J. Dairy Res.* **59** (1992) 57
3. J. L. Weihrauch, C. R. Brewington, D. P. Schwartz, *Lipids* **9** (1974) 883
4. W. R. Mayberry, *J. Bacteriol* **147** (1981) 373
5. M. A. Loneelle, J. C. Prome, *J. Gen. Microbiol.* **136** (1990) 773
6. P. M. Teresa, S. Elisoe, T. Amparo, *J. Nat. Prod.* **47** (1984) 184
7. H. Hughes, C. V. Smith, E. C. Horning, J. R. Mitchell, *Anal. Biochem.* **130** (1983) 431
8. D. T. Downing, Z. H. Kranz, J. A. Lamberton, K. E. Murray, A. H. Redcliffe, *Australian J. Chem.* **14** (1961) 253
9. R. John, *Organic Syntheses* **4** (1963) 145
10. A. L. Wilds, *Organic Reactions* **2** (1944) 178
11. H. Çelik, S. Özeriş, *Chim. Acta Turc.* **24** (1996) 23
12. A. Yusufoglu, H. Çelik, *Chim. Acta Turc.* **24** (1996) 29
13. R. T. Holman, W. D. Lundberg, T. Malkin, *Progress in the Chemistry of Fats and Other Lipids* **3** (1955) 243
14. G. F. Townsend, W. H. Brown, *Can. J. Biochem. Physiol.* **39** (1961) 1765
15. S. Tolnai, J. F. Morgan, *Can. J. Biochem. Physiol.* **40** (1962) 1367
16. J. N. Cardoso, G. Eglinton, *Geochim. Cosmochim. Acta* **47** (1983) 723
17. A. M. Milton, A. E. Axelrod, K. Hofmann, *J. Am. Chem. Soc.* **72** (1950) 1231
18. H. P. Kaufmann, W. Stamm, *Chem. Ber.* **91** (1958) 2121
19. M. Asano, Y. Kamedo, T. Wada, *J. Pharm. Soc. Japan* **64/8A** (1944) 24
20. S. Özeriş, *Fette, Seifen, Anstrichmittel* **63** (1961) 805
21. F. L. Breusch, A. Kirkali, *Fette Seifen Anstrichmittel* **67** (1965) 4
22. M. Skogh, *Acta Chem. Scand.* **6** (1952) 809
23. N. G. Gaylord, *Reduction with Complex Metal Hydrides*, Interscience, New York, 1956
24. E. Schenker, *Neuere Methoden der Preparativen Organischen Chemie* **4** (1966) 447
25. A. I. Vogel, *Practical Organic Chemistry*, Longmans, Green and Co Ltd., 3<sup>rd</sup> Edition, 1961, p. 367

26. E. E. Blaise, C. R. Hebd, *Seacens Acad. Sci.* **157** (1913) 1440
27. A. I. Vogel, *Practical Organic Chemistry*, Longmans, Green and Co Ltd., 3<sup>rd</sup> Edition, 1961, p. 938
28. A. I. Vogel, *Ibid*, 1961, p. 3
29. L. Shriner, R. C. Fuson, D. Curtin, *The Systematic Identification of Organic Compounds*, John Wiley, New York, 1964.