Applicability of the Turbidity Test for Castor Oil to Some Other Hydroxy and Epoxy Fatty Compounds

GOLLAMUDI LAKSHMINARAYANA, Regional Research Laboratory, Hyderabad-9, India

Abstract

A large number of hydroxy as well as nonhydroxy fatty compounds were employed to explore the applicability of the turbidity test for castor oil (1) and to understand the nature of the turbidity. The test was capable of detecting up to ca. 2% of hydroxy, hydroperoxy, and epoxy fatty acids and their esters, also the epoxy, acetoxy, and hydroxy fatty alcohols present in a petroleum ether solution of a nonpolar triglyceride oil. Monohydric primary alcohols, sterols, mono- and diglycerides were not detected. The turbidity developed, whether H_2SO_4 contained ammonium molybdate or not, because of the formation of a sulfate and its precipitation from the petroleum ether solution of oil.

Introduction

KUMAR (1) PROPOSED A TEST for the detection of castor oil in other oils, which noted the development of pearly white turbidity on the addition of a drop of H_2SO_4 solution of ammonium molybdate to an acidified petroleum ether solution of oil. The nature of the turbidity was not explained. While this paper was in preparation, Ahmed et al. (2) examined the response of a variety of oils to the test but have not put forward a mechanism which explains the cause of the turbidity. To understand this and to explore the scope of the test, a number of hydroxy and nonhydroxy fatty compounds were tested and the results are reported.

Experimental Procedures

The compounds tested (Table I) were pure as judged by thin-layer and/or gas-liquid chromatography, and the oils were genuine.

Only the upper layer was used of petroleum ether (40–60°), acidified with 2% (by volume) of HCl (A. R., sp gr 1.19). Ammonium molybdate (A.R., M_0O_3 81%) solution (1.25%) in H_2SO_4 (A.R., sp gr 1.84) or H_2SO_4 alone was the other reagent.

Petroleum ether (10 ml) was added to the compound (ca. 10 mg) and shaken. Compounds insoluble in petroleum ether were dissolved in a minimum volume (0.05–0.2 ml or 1–4 drops) of *n*-butanol, which did not interfere with the test, prior to the addition of the reagent. To the clear solution thus obtained a drop (ca. 0.05 ml) of the other reagent was added without shaking, and the development of turbidity was immediately noted. Since castor oil and many other hydroxy compounds did not become turbid when tested in this way, the test was repeated after solubilizing the material (ca. 10 mg) in one ml of refined cottonseed oil (CSO) or any other common oil which responds negatively to the test. A minimum volume of *n*-butanol was used when necessary. As H_2SO_4 alone will give a turbidity with samples adulterated with castor oil (3), the test was also carried out by using H_2SO_4 with no ammonium molybdate. Of all the materials tested, no significant difference in response was noted whether H_2SO_4 contained ammonium molybdate or not. The results are given in Table I.

Discussion

Sulfation appears to be essential in causing turbidity since only the use of either H_2SO_4 (90–98%) or chlorosulfonic acid resulted in turbidity when the samples were adultered with castor oil whereas the use of HCl, HNO_3, H_3PO_4, and saturated aqueous ammonium molybdate solution did not. Turbidity resulted, only on shaking, even with 50–80% H_2SO_4 but not with acid of lesser concentration. That sulfation occurs under the test conditions to a considerable extent, though not completely, was proved by the high SO_3 content, 4.3% as against 5.7% for sulfated castor oil (4,5), of the material causing the turbidity when isolated in the following manner.

The test was carried out on several mixtures of 10% castor oil in coconut oil. The turbid upper layers of petroleum ether were immediately filtered under vacuum, through a sintered glass filter, by decanting from the lower H_2SO_4 layer. The combined insoluble matter on the filter was washed with petroleum ether to remove coconut oil. Water and ethanol were then added in succession, and the filtrate was collected in a separate receiver and neutralized. After removal of the solvent, the dry residue was extracted with *n*-butanol saturated with NaCl. The butanol extract was filtered, and the solvent was evaporated off thoroughly before determining the organically combined SO_3 content (4).

For sulfation to occur, there had to be present in the compound at least one hydroxy, hydroperoxy, or epoxy group capable of forming a sulfate since compounds which do not contain these groups or in which the hydroxy group is esterified, as in methyl 12-acetoxystearate and ergot oil (6), did not become turbid (Table I). Methyl oleate, which was mildly autoxidized at room temperature for a month by exposure to diffused daylight, became turbid, but a methyl oleate sample which was highly peroxidized in direct sunlight did not, presumably because of the presence of hydroperoxides in the former and their destruction in the latter.

Epoxy fatty acids and *Vernonia anthelmintica* seed oil containing ca. 70% of 12,13-epoxyoleic acid but no hydroxy acid (7) gave turbidity even without prior acidification of petroleum ether with HCl, showing that the opening of the epoxy ring and subsequent sulfation readily occurred on the addition of H_2SO_4. Incidentally HCl did not play a part in the development of turbidity with any sample: it only delayed charring of oil (1,2).

The development of turbidity also seemed to depend on the facility and consequent rapidity of sulfation, which in turn depended on the polarity of the solvent medium. In petroleum ether, ready sulfation occurred of high-melting palmityl and stearyl alcohols and 1,3-distearin; these were sufficiently nonpolar because of the long alkyl chains and single OH group present in each, but short- and medium-chain alcohols (viz., hexanol and myristyl alcohol) as well the more polar compounds (viz.,

TABLE I

Response of Fatty Compounds to H_2SO_4 With or Without Ammonium Molybdate

No turbidity either as such or in cottonseed oil	Turbidity as such but not in cottonseed oil	Turbidity in cottonseed oil but not as such		
n-Hexadecane	Alcohols	Acids	Methyl esters	Alcohols
Fatty acids	1 OH 16:0	6(7) OH 18:0	18:1 mildly autoxidized	1,6(7) OH 18:0
4:0 to 22:0[a]	1 OH 18:0	9(10) OH 18:0	12 OH 18:0	1,9(10) OH 18:0
Methyl esters	1,3-Distearin	13(14) OH 22:0	18 OH 18:0	1, 12 OH 18:0
12:0 to 16:0	*A. mexicana* oil	18 OH 18:0	12 OH 18:1	1, 12 OH 18:1
11:0		12 OH 18:1	*erythro*-9,10 OH 18:0	1,13(14) OH 22:0
18:1		*threo*-6,7 OH 18:0	*threo*-9,10 OH 18:0	1,18 OH 18:0
18:1 Highly autoxidized		*erythro*-9,10 OH 18:0	9(10) Keto, 10(9) OH 18:0	1 OH 9,10 epoxy 18:0
12 Acetoxy 18:0		*threo*-9,10 OH 18:0	Glycerides	1,10,11 OH 11:0
12 Keto 18:0		*threo*-13,14 OH 22:0	Mono-, di-, and	1,9,10 OH 18:0
Ethylene glycol dipalmitate		*erythro*-9,10,12 OH 18:0	triricinoleo-	*threo*-1,13,14 OH 22:0
Glycerides		*threo*-9,10,12 OH 18:0	triglycerides	1,9,10,12 OH 18:0
1-Monostearin		9(10) Keto, 10(9) OH 18:0	Castor oil	
1,3-Lauropalmitin		6,7 Epoxy 18:0	*V. anthelmintica* oil	
Tripalmitin		9,10 Epoxy 18:0	1 Acetoxy, 13(14) OH 22:0	
Ergot oil		13,14 Epoxy 22:0		
Kamalaseed oil				
Peanut oil				
phospholipids				
Alcohols				
1 OH 6:0 to 1 OH 14:0				
1 OH 11:0				
1 OH 11:1				
1 OH 18:1				
Sterols				
Cholesterol				
Ergosterol				

[a] Figure before colon indicates carbon chain-length and after colon the number of double bonds. The position of the double bond is at 9th position for 18:1 and at 10th for 11:1. The configuration is of *cis* where applicable. The figure before the functional group indicates its position in the chain. Examples of designation are 12 OH 18:1, ricinoleic acid or ester; 1 OH 18:1 oleyl alcohol; and 1 acetoxy 13(14) OH 22:0, 13(14)-hydroxydocosanol-1-acetate.

hydroxy acids, esters, and alcohols, 1-monostearin and 1,3-lauropalmitin) were not sulfated. Even a petroleum ether solution of decanol became turbid on shaking with H_2SO_4, and this turbidity disappeared on further shaking with either water or an aqueous KOH solution. Methyl 12-hydroxystearate in petroleum ether did not give a turbidity even on shaking although the H_2SO_4 turned turbid and yellow. On the addition of water and further shaking an emulsion resulted; a sulfate was formed which was more soluble in H_2SO_4 than in petroleum ether. In a petroleum ether solution of CSO, decanol showed no turbidity. When this clear solution was added to water, taking care that the H_2SO_4 remained behind, and was then shaken, an emulsion resulted which proved that a sulfate was formed. Emulsions were not formed in blank experiments.

The polarity of the oil vehicle was also important. Methyl 12-hydroxystearate did not give a turbidity when solubilized in medicinal paraffin. But a triglyceride oil brought together the nonpolar petroleum ether, polar compound, and H_2SO_4 to facilitate sulfation.

Further, the solubility of the sulfate formed in the petroleum ether solution of the oil was also a crucial factor in causing turbidity. For example, the turbid petroleum ether solutions, obtained by the addition of H_2SO_4 to stearyl alcohol and 1,3-distearin as well as the solution obtained from decanol on shaking, became clear when added to CSO. Sulfates from more polar compounds, e.g., methyl 12-hydroxystearate, were readily thrown out from a petroleum ether solution of oil, causing turbidity.

Concentration of the polar compounds in oil was also important. Castor and *V. anthelmintica* oils did not give turbidity as such, presumably owing to the solubility of sulfates in the strongly polar media but gave turbidity after dilution with CSO. *Argemone mexicana* oil, which contained ca. 2% of epoxy and hydroxy acids (8), gave turbidity only as such, and not after mixing with CSO, because of the insufficient sensitivity of the test. A sample of unknown composition should therefore be tested as such and after solubilizing in a suitable proportion of nonpolar oil.

Even when the conditions are satisfactory for the development of turbidity, the test may fail, as seen with kamalaseed oil on account of extremely rapid charring.

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REFERENCES

1. Kumar, R., JAOCS *40*, 80 (1963).
2. Ahmed, S. A., D. Ramachar, B. A. K. Somayajulu and S. D. T. Rao, Oils and Oilseeds J. (Bombay) *19*(9), 8 (1967).
3. Private communication from B. P. Oil Mills, Agra.
4. Official and Tentative Methods of the AOCS, 2nd ed., rev. to 1960, AOCS, Chicago, Ill., Method F2a-44.
5. Geoghegan, J. T., C. O. Myatt and M. Rodson, JAOCS *43*, 600 (1966).
6. Morris, L. J., and S. W. Hall, Lipids *1*, 188 (1966).
7. Miwa, T. K., F. R. Earle, G. C. Miwa and I. A. Wolff, JAOCS *40*, 225 (1963).
8. Mani, V. V. S., and G. Lakshminarayana, unpublished work.

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