391. *The Spectrophotometric Determination of the Hydroxyl Contents of Aliphatic and Alicyclic Alcohols.*

By J. P. Riley.

A rapid spectrophotometric method for the determination of the hydroxyl content of aliphatic and alicyclic alcohols is described. The hydroxy-compound is esterified with β-2 : 4-dinitrophenylpropionyl chloride. From the extinction coefficient of the recovered ester at 242 mμ, the acetyl value of the original alcohol can be calculated. Thirteen β-2 : 4-dinitrophenylpropionates are described. The method is not suitable for hydroxy-compounds showing appreciable absorption at 242 mμ, but a procedure is suggested for use with such compounds. Results 5·4% lower than theoretical are obtained with castor oils, perhaps owing to steric effects, but by the use of an empirical correction factor, the procedure can be used for their evaluation.

Of the methods proposed for the determination of acetyl values, two adopted by the B.S.I. (Specification 684, 1950) are most frequently used. The first (*loc. cit.*, p. 42; see also Riley, *Analyst*, 1951, **76**, 41) depends on acetylation of the hydroxy-compound with boiling acetic anhydride, followed by isolation of the acetyl compound and determination of the acetic acid obtained from it by hydrolysis; this procedure, though accurate, is very time-consuming and is not suitable for work with small samples. The second (*loc. cit.*, p. 44), which depends on acetylation by means of acetic anhydride–pyridine and subsequent determination of the acetic anhydride consumed, is rapid and requires only small amounts of material; it suffers, however, from the defect that the end-point of the titration is by no means sharp.

In an endeavour to devise an accurate method requiring only small weights of sample, it was thought that the hydroxyl group might be esterified with some chromophoric group, the resultant derivative isolated, and the proportion of the group in it determined spectrophotometrically. Earlier experiments using 3 : 5-dinitrobenzoyl chloride as esterifying agent were unsuccessful, since the absorption band of the ester moved in the direction of shorter wave-length as the molecular weight of esterified alcohol increased; *e.g.*, the absorption bands of the esters of methyl alcohol, ethyl alcohol, and methyl 12-hydroxystearate are at 230, 223, and <210 mμ, respectively. This effect was thought to be due to conjugation of the carboxyl group with the chromophore, and it was considered that if these two groups were separated by one or more methylene groups, then the position of the absorption band might not alter whatever the molecular weight of the esterifying alcohol. Experiments were therefore made using as esterifying agent the readily prepared β-2 : 4-dinitrophenylpropionyl chloride, which reacts in the cold with liquid alcohols, primary, secondary, and tertiary. For every ester examined $\lambda_{\text{max.}} = 242$ and $\varepsilon_{\text{max.}} = 14,443 \pm 0{\cdot}7\%$. There was a minimum at *ca.* 210 mμ ($\varepsilon \simeq 11,200$) and the absorbency fell off to a low value above 330 mμ. Since the molecular extinction coefficient of all the esters was the same, the amount of the β-2 : 4-dinitrophenylpropionyl radical present in the ester could be determined by measurement of its absorption coefficient at 242 mμ, and from this the acetyl value of the alcohol could be readily calculated.

The method proposed therefore consists of heating the alcohol under examination with excess of the acid chloride. After reaction has occurred, the remaining acid chloride is hydrolysed with boiling water, the ester recovered by extraction with ether, and free acid washed out with alkali. The absorption coefficient of the recovered ester at 242 mμ is determined in duplicate. Thirteen alcohols have been examined by the proposed method, mostly in duplicate, with the results shown in Table 1; in all cases the molecular extinction coefficients given are the mean of at least four determinations.

A high but variable yield of ester is obtained by the reaction of dinitrophenylpropionyl chloride with the alcohols examined. This variation is not due to the incompleteness of reaction but rather to the difficulty of ensuring complete extraction of the comparatively small quantities of ester obtained. The particularly low recovery of the ester of methyl tetrahydroxystearate is caused by its low solubility in ether.

TABLE 1

Ester	Yield, %	M. p.	n_D^{40}	ϵ †	$E^{1\%}_{1\,cm.}$ ‡ for acyl group §	Formula	N, %: Calc.	Found
Butyl	90·7	—	1·5268	14,550	608·8	$C_{13}H_{16}O_6N_2$	9·46	9·50
Amyl	97·5	—	1·5220	14,450	604·6	$C_{14}H_{18}O_6N_2$	9·03	9·00
Octyl	89·3	—	1·5118	14,450	604·6	$C_{17}H_{24}O_6N_2$	7·96	7·81
sec.-Octyl	92·3	—	nd	14,420	603·3	$C_{17}H_{24}O_6N_2$	7·96	7·90
Dodecyl	94·2	32°	1·4998	14,520	607·5	$C_{21}H_{32}O_6N_2$	6·86	6·72
Tetradecyl	98·9	38	1·4975	14,360	600·8	$C_{23}H_{36}O_6N_2$	6·42	6·50
*cyclo*Hexyl	86·6	—	1·5372	14,360	600·8	$C_{15}H_{18}O_6N_2$	8·69	8·74
Methyl*cyclo*hexyl	92·1	—	1·5352	14,330	599·5	$C_{16}H_{20}O_6N_2$	8·33	8·13
(+)-Bornyl	95·3	—	1·5360	14,500	606·7	$C_{19}H_{24}O_6N_2$	7·45	7·53
Methyl hydroxystearyl	96·6	—	1·4950	14,600	610·9	$C_{28}H_{44}O_8N_2$	5·22	5·01
Methyl ricinoleyl	95·1	—	1·5006	14,320	599·2	$C_{28}H_{42}O_8N_2$	5·24	5·22
Methyl dihydroxystearyl	98·0	19	1·5188	29,690	621·1	$C_{37}H_{50}O_{14}N_4$	7·23	7·15
Methyl tetrahydroxystearyl	67·6	*	1·5415	58,980	616·9	$C_{55}H_{62}O_{26}N_8$	8·96	8·75
		|| Mean		14,443	604·3			
		Mean deviation			0·53%			
		Standard deviation			0·68%			

* Glassy.
† Molecular extinction coefficient (expressed in g.-mol./l.) at 242 mμ.
‡ At 242 mμ. § Acyl = β-2 : 4-dinitrophenylpropionyl.
|| Excluding di- and tetra-hydroxystearyl esters.

The esters themselves, with three exceptions, are light yellow, rather viscous oils or glassy solids at room temperature, and seem to be quite stable for at least six months under these conditions. They blacken, however, if heated above 100° for any length of time, and overheating during their preparation should be avoided.

The wave-length of maximum absorption of all the esters lies at the same value, *viz.*, 242 mμ, and the molecular extinctions of all the esters of the monohydric alcohols are the same within the limits of experimental error (see Table 1). Those of the esters of methyl di- and tetra-hydroxystearate are, however, respectively 2·8% and 2·1% higher than the mean value (after division of the molecular extinction by the number of dinitrophenylpropionyl groups in the molecule); this augmentation of the absorption may perhaps be associated with steric effects, since the chromophoric groups in these compounds are very close together.

It is, of course, important that the alcohol under examination should not show any selective absorption at 242 mμ, and the method cannot therefore be applied to aromatic compounds. It is possible that it might be applied to such compounds by making use of the brown colour formed by the action of alcoholic potassium hydroxide on the dinitrophenylpropionyl group in the ester.

TABLE 2

	Characteristics of dinitrophenylpropionyl compound				Chemically † determined	
Source of oil	n_D^{40}	$E^{1\%}_{1\,cm.}$ *	Mean	Ac Val.	Ac Val.	Error, %
Tobago	1·5120	255·0, 253·4	254·2	144·5	152·8	−5·4
Tanganyika (Central)	1·5110	255·1, 255·8	255·4	145·5	152·8	−4·8
East Africa	1·5106	251·1, 250·8	250·9	141·8	149·3	−5·0
Nigeria (large)	1·5102	250·3, 254·1	252·1	142·8	150·6	−5·2
Nigeria (medium)	1·5085	246·2, 245·0	245·6	137·5	147·4	−6·8
					Mean	−5·4

* At 242 mμ. † Gupta *et al.*, *loc. cit.*

The dinitrophenylpropionyl chloride is unreactive toward ethylenic unsaturation, and it did not react with either methyl oleate or methyl linoleate. Methyl ricinoleate gives theoretical results for reaction with the hydroxyl group only, showing that no reaction with the double bond occurs.

Attempts were made to apply the method to the determination of acetyl values of alkali-refined castor oils (Gupta, Hilditch, and Riley, *J. Soc. Food Agric.*, 1951, **2**, 245), with the results shown in Table 2, from which it will be seen that results about 5·4% low are consistently obtained with castor-oil glycerides, possibly owing to steric hindrance having prevented complete reaction of the glyceride with the rather large dinitrophenylpropionyl group. If, however, a correction factor of 1·057 is applied, then the method may be used for the evaluation of castor oils.

EXPERIMENTAL

Preparation of β-2 : 4-*Dinitrophenylpropionic Acid* (cf. Gabriel and Zimmermann, *Ber.*, 1880, **13**, 1681).—β-Phenylpropionic acid (100 g.), prepared by hydrogenation of cinnamic acid, was dissolved in fuming nitric acid (660 ml.) and cooled to 0°; concentrated sulphuric acid (360 ml.) was added gradually with constant stirring and cooling in ice. After an hour (at room temperature) the mixture was poured on crushed ice (1·5 kg.). The yellow crystals of crude dinitrophenylpropionic acid (95 g.) were filtered off, washed well with cold water, and thrice recrystallised from boiling water (Norite), yielding the pure acid (71 g.; m. p. 127·7—128·1°; $\varepsilon = 13{,}000$ at 242 mμ) as long pale yellow needles.

β-2 : 4-*Dinitrophenylpropionyl Chloride.*—The acid (15 g.) was treated with thionyl chloride (10 ml.; freshly distilled from linseed oil) and refluxed in an oil-bath at 100° until homogeneous (*ca.* 1 hour). Excess of thionyl chloride was removed by distillation, and the last traces were removed *in vacuo* at 100°. The acid chloride, a yellow viscous liquid obtained in theoretical yield, was preserved in a well-corked flask kept in a desiccator.

Alcohols used.—Butyl and amyl alcohol were pure specimens supplied by the Bureau international des étalons physico-chimiques. Dodecyl and tetradecyl alcohol (m. p. 23·8° and 37·5°, respectively) were prepared by sodium and alcohol reduction of Dika fat and fractionation of the resultant alcohols. Methyl 12-hydroxystearate, ricinoleate, oleate, and linoleate were prepared in these laboratories (Riley, *loc. cit.*). Methyl 9 : 10-dihydroxystearate (m. p. 111·5—112°) was prepared by esterification of 9 : 10-dihydroxystearic acid (m. p. 141°) isolated from castor oil. Methyl 9 : 10 : 12 : 13-tetrahydroxystearate was prepared by esterification of tetrahydroxystearic acid (m. p. 171°), produced by alkaline permanganate oxidation (Lapworth and Mottram, *J.*, 1925, **127**, 1628) of a concentrate of linoleic acid and removal of the isomeric tetrahydroxystearic acid, m. p. 157°, also formed. The other alcohols used were good commercial specimens which had been carefully fractionated.

Method.—The hydroxyl compound (0·1—0·4 g.) is weighed into a 6″ × 1″ Pyrex boiling-tube, about twice the theoretical quantity of β-2 : 4-dinitrophenylpropionyl chloride added, and the tube is loosely stoppered with a Kjeldahl stopper. The mixture is heated to 90° for 1 minute with continual shaking, the onset of the reaction being indicated by the evolution of copious fumes of hydrogen chloride. The product is poured into warm water (40 ml.) and heated on the water-bath for 20 minutes to hydrolyse the excess of acid chloride; after cooling, N-sodium carbonate (2 ml.) is added, and the solution extracted twice with ether. In order to obtain high recoveries of the ester, the reaction tube should be filled with hot water, and, after cooling, washed out with ether; both water and ether are added to the main extraction. In no circumstances should the hydrolysis of the acid chloride be carried out in the reaction tube, since the resultant ester is much darkened under these conditions. The free β-2 : 4-dinitrophenylpropionic acid is washed out from the ethereal layer by three extractions with 10-ml. portions of N-sodium carbonate solution, and washed with water until neutral. The ethereal extract is dried (Na_2SO_4), and the ether distilled off, the last traces being removed *in vacuo* at a temperature not exceeding 80°. The extinction coefficient of the product in solution in ethyl alcohol is determined at 242 mμ. If the ester is not readily soluble in alcohol (as in the case of the esters of tetrahydric alcohols), it may be dissolved in 2 ml. of ether and then made up to volume with alcohol.

Calculation.—If the extinction coefficient ($E^{1\%}_{1\,\text{cm.}}$) of the ester at 242 mμ is x, then the percentage of β-2 : 4-dinitrophenylpropionyl group in the ester, *viz.*, $y = 100x/604{\cdot}3$, and the acetyl value $= 5610y/(2390—18y)$, and hydroxyl $= 1700y/(23{,}900—222y)\%$.

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