

Methods for the Isolation and Characterization of <sup>Official Use</sup>  
Constituents of Natural Products

XIV. Use of Iodine Monochloride for Detecting Unsaturation  
in Microgram Quantities of Colored Derivatives

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The detection of unsaturation in pure compounds isolated from natural products is an important prerequisite to their identification. At the microgram level, the noninstrumental methods which are currently favored in this regard include thin-layer adsorption chromatography on  $\text{AgNO}_3$ -impregnated plates (6) and bromination (2-4) also on thin-layer plates. For colored derivatives, principally 2,4-dinitrophenylhydrazones, only the  $\text{AgNO}_3$  method has been extensively investigated (1, 3, 5, 8). Although this technique has been used with gratifying results in this laboratory, there have been instances when we were not always certain that a double bond existed based solely on the mobility of the unsaturated compound on a thin-layer plate impregnated with  $\text{AgNO}_3$ . An investigation of classical reagents for detecting double bonds has revealed that iodine monochloride is an efficient and relatively mild reagent for accomplishing this. A comprehensive study of the use of this reagent for the detection of unsaturation in colored derivatives of alcohols, carbonyls, amines, and acids is described below.

REAGENTS AND APPARATUS <sup>2</sup>

A 1% (w:v) solution of iodine monochloride (ICl) (Eastman Kodak Co., Rochester, N.Y. in  $\text{CCl}_4$ ; melting point capillaries open at both ends (approx 1.5-2.0 mm o.d. and 100 mm long (Fisher Scientific Co., Silver Spring, MD, cat. no. 12-141). Thin-layer partition plates prepared according to Schwartz and Brewington (7) except that

<sup>1</sup> Eastern Marketing and Nutrition Research Division, Agricultural Research Service, U. S. Department of Agriculture.

<sup>2</sup> Mention of specific commercial products does not imply endorsement.

1 ml of 85%  $\text{H}_3\text{PO}_4$  was added to the slurry to stabilize the layer against flaking.

#### EXPERIMENTAL METHODS

Bend the capillary approximately at its center to an angle of 120–150°. Dip one end into the solution of ICl so that a column of liquid approximately 2–4 mm is retained in the capillary. Allow the liquid to fall to the bend and inject 1–3  $\mu\text{l}$  of a  $\text{CCl}_4$  solution of the derivative into the same end. Tilt the capillary so that the two solutions will mix. Immediately spot the contents directly from the capillary onto an area adjacent to a spot containing untreated derivative on a thin-layer partition plate and develop the plate immediately in an appropriate solvent. In most instances the effect of the addition of ICl to the double bond is apparent after the solvent has ascended a short distance and the plate can be removed. Exposure of the plate to diethylamine vapor or spraying with 1 *N* methanolic KOH can be used, if necessary, to intensify the spots.

#### RESULTS AND DISCUSSION

Table 1 lists the colored derivatives that were investigated. Included in the study were 2,4-dinitrophenylhydrazones, the 2,6-dinitrophenylhydrazone derivatives of pyruvic acid esters of alcohols, the  $\alpha$ -methyl-2,4,6-trinitrophenylhydrazone derivatives of alkoxyacetaldehyde compounds, *N*-2,4-dinitrophenylamine derivatives and amides of pyruvic acid 2,6-dinitrophenylhydrazone. The percentage retardation was calculated from the respective  $R_f$  values of the treated and untreated derivatives. Approximately 1–5  $\mu\text{g}$  of the derivative was used. However,  $6 \times 10^{-4}$   $\mu\text{moles}$  of an alcohol derivative and  $7 \times 10^{-4}$   $\mu\text{moles}$  of a 2,4-dinitrophenylhydrazone can still be halogenated and the spot detected on exposure of the plate to diethylamine vapor or by spraying with 1 *N* methanolic KOH.

It was first established that saturated compounds are not retarded when exposed to ICl. A large number of saturated derivatives (both carbonyls and alcohols) were subjected to the ICl procedure but not all of these are listed in Table 1. There is some destruction of both saturated and unsaturated 2,4-dinitrophenylhydrazone derivatives of carbonyl compounds, but not of the alcohol derivatives. This destruction is manifested by a diminution in the intensity of the color of the spot of the treated compound. As a consequence, treatment of 2,4-dinitrophenylhydrazone derivatives of carbonyl compounds with ICl should be carried out as rapidly as possible.

In all instances, where addition of ICl to the double bond took place,

TABLE 1  
RETARDATION OF COLORED DERIVATIVES ON THIN-LAYER PARTITION PLATES  
AFTER REACTION WITH IODINE MONOCHLORIDE

	No. of double bonds	Retardation (%) in	
		Hexane	Hexane:benzene (65:35)
Alcohols (as esters of pyruvic acid 2,6-dinitrophenylhydrazone)			
stearyl	0	0	
elaidyl	1	17	
vaccenyl	1	16	
oleyl	1	20	
linoleyl	2	51	
linolenyl	3	79	
11,14-eicosadien-1-ol	2	40	
cholestanol	0	0	
cholesterol	1	25	
lathosterol	1	0	
dihydrolanosterol	1	32	
lanosterol	2	67	
9,10-epoxystearyl	0	0	
10-undecen-1-ol	1		23
phytol	1		9
L-menthol	0		0
citronellol	1		13
L-isopulegol	1		42
linalool	2		54,74,83 <sup>a</sup>
farnesol	3		52
$\beta$ -phenylethanol	0		0
benzyl	0		0
3-phenyl-propan-1-ol	0		0
cinnamyl	1		20
furfuryl	2		92 <sup>b</sup>
4-penten-1-ol	1		40
1-penten-3-ol	1		32
4-penten-2-ol	1		40
3-penten-2-ol	1		33
2-methyl-2-penten-1-ol	1		22
2,4-hexadien-1-ol	2		26
1,4-pentadien-3-ol	2		31,53 <sup>c</sup>
1,6-heptadien-4-ol	2		44,93 <sup>c</sup>
<i>cis</i> -3-hexen-1-ol	1		29
4-hexen-1-ol	1		29
4-hexen-3-ol	1		30
2-octen-1-ol	1		16
allyl	1		26
ricinoleyl	1		22 <sup>d</sup>

TABLE 1 (continued)

	No. of double bonds	Retardation (%) in	
		Hexane	Hexane-benzene (65:35)
arboyls (as 2,4-dinitrophenylhydrazones)			
stearyl	0	0	
oleylaldehyde	1	14	
2-hexadecenal	1	0	
tiglaldehyde	1		0
acrolein	1		0
2-ethyl-2-hexenal	1		0
<i>trans</i> -3-hexenal	1	0	0
<i>cis</i> -3-hexenal	1		60
<i>cis</i> -4-hexenal	1		50
<i>cis</i> -4-heptenal	1		47
<i>cis</i> -5-heptenal	1		38
7-octenal	1		33
nonanal	0		0
2-nonenal	1		0
<i>trans</i> -4-nonenal	1		36
<i>trans</i> -5-nonenal	1		36
<i>cis</i> -6-nonenal	1		28
<i>trans</i> -6-nonenal	1		23
<i>trans</i> -7-nonenal	1		33
8-nonenal	1		29
<i>cis</i> -7-decenal	1		31
2,4-octadienal	2		0
2,4-hexadecadienal	2		0
<i>trans</i> -2- <i>trans</i> -6-nonadienal	2		34
<i>trans</i> -2- <i>cis</i> -6-nonadienal	2		36
<i>trans</i> -2- <i>cis</i> -7-decadienal	2		34
<i>cis</i> -4- <i>cis</i> -7-decadienal	2		50
<i>trans</i> -2- <i>cis</i> -5-undecadienal	2		22
<i>cis</i> -5- <i>cis</i> -8-tetradecadienal	2	69	
2-ethyl-2-butyl-5-methyl-3,4-hexadienal	2		77
<i>trans</i> -2- <i>cis</i> -6- <i>cis</i> -9-pentadecatrienal	3	70	
<i>trans</i> -2- <i>cis</i> -5- <i>cis</i> -8-tetradecatrienal	3	67	
glycolaldehyde stearate	0	0	
glycolaldehyde oleate	1	35	
glycolaldehyde linoleate	2	81	
glycolaldehyde linolenate	3	86	
2-heptadecanone	0	0	
acetone	0		0
cholestan-3-one	0	0	
methyl-12-ketostearate	0	0	
ethyl pyruvate	0		0
mesityl oxide	1		0
1-hepten-4-one	1		0

TABLE 1 (continued)

	No. of double bonds	Retardation (%) in	
		Hexane	Hexane:benzene (65:35)
2-nonen-4-one	1		0
testosterone	1		0
5-cholesten-3-one	1	0	
phorone	2		0
Alkoxyacetaldehyde compounds (as $\alpha$ -methyl-2,4,6-trinitrophenylhydrazones)			
octadecyloxyacetaldehyde	0	0	
9-octadecenyloxyacetaldehyde	1	30	
9,12-octadecadienyloxyacetaldehyde	2	58	
Amines (as amides of pyruvic acid 2,6-dinitrophenylhydrazone)			
decyl	0		0
aniline	0		0
oleyl	1	20	
allyl	1		30
skatole	1		63
Acids (as <i>N</i> -2,4-dinitrophenyl-ethanolamine esters)			
lauric acid, ethanolamine ester	0	0	
palmitoleic acid, ethanolamine ester	1		20

<sup>a</sup> Three spots after treatment with ICl.

<sup>b</sup> Plate developed with hexane:benzene (2:1) saturated with stationary phase.

<sup>c</sup> Two spots after treatment with ICl.

<sup>d</sup> Plate developed with hexane:benzene (85:15). Ricinoleyl alcohol was investigated as the bis-derivative. This has zero  $R_f$  in hexane and the treated compound moves the same in hexane:benzene (65:35).

a sufficiently significant reduction occurred in the mobility of the treated derivative relative to the untreated derivative to warrant a conclusion regarding the unsaturated nature of the compound. Moreover, the complete absence of any unreacted derivative was always observed which helps to simplify the conclusion of whether a double bond is present. Preliminary experiments which involved spotting the derivative at the origin of a thin-layer plate and overspotting with the ICl solution always failed to completely halogenate the derivative. Despite the fact that the reaction had gone to a sufficient extent to detect a slower-moving component besides the original derivative, this approach was considered unsatisfactory because of the possibility of an unknown having zero mobility after halogenation. In these instances one might erroneously conclude that the compound was saturated.

A number of unsaturated derivatives failed to add ICl. All alk-2-enals, alk-2,4-dienals, and all  $\alpha,\beta$ -unsaturated ketones were unchanged after treatment. The 2,4-dinitrophenylhydrazones of testosterone and  $\Delta^5$ -cholesten-3-one also failed to react. *Trans*-3-hexenal (but not the *cis*-isomer) and 1-hepten-4-one likewise could not be halogenated. Thus, it appears that carbonyl compounds containing a single double bond or two double bonds conjugated with the hydrazone linkage and certain 2,4-dinitrophenylhydrazones containing a  $\beta, \gamma$ -bond will not react with ICl under the specified conditions. All unsaturated alcohol derivatives reacted satisfactorily except lathosterol ( $\Delta^7$ -cholesten-3 $\beta$ -ol).

In some instances, in a given solvent system, derivatives containing more than one double bond are retarded to a significantly greater extent than are derivatives containing only one double bond. Thus, the derivatives of linoleyl and linolenyl alcohols and the glycolaldehyde esters of linoleic and linolenic acids are retarded to a greater extent than are oleyl and elaidyl alcohols and glycolaldehyde oleate, respectively. Similarly, lanosterol which differs from dihydrolanosterol by having a double bond in the side chain is retarded twice as much. Unfortunately, there were a number of exceptions. In the alcohols, 2,4-hexadien-1-ol is retarded as if it were a monoene. 1,6-heptadien-4-ol, and 1,4-pentadien-3-ol each gave two spots, one each of which was retarded like a monoene, the other as if it were a diene, suggesting that the addition of the halogen was incomplete for the second double bond.

The double bond in the pyrrolyl nucleus of skatole presumably reacted since the double bonds of the benzene ring are apparently inert to ICl under the prescribed conditions. At least one of the double bonds in furfuryl alcohol also reacted.

In several cases it was observed that derivatives which had added ICl to the double bond gave a different color than did the untreated derivative when the chromatogram was exposed to diethylamine vapor. This was especially true with the derivatives of alcohols which gave a reddish hue for the halogenated derivatives as opposed to a violet color for the untreated derivative. This color difference facilitated the analysis of ricinoleyl alcohol which was investigated as the *bis*-derivative. This compound, after treatment with ICl, moved identically to the untreated compound in hexane: benzene (65:35). Exposure of the plate to diethylamine vapor revealed the color difference. Separation was then effected in hexane: benzene (85:15).

A few trials were carried out to determine whether adsorption chromatography on silica gel G plates would effect significant separation of treated and untreated derivatives. However, the differences in

$R_f$  were usually less than 5% and further study of this system was abandoned.

#### SUMMARY

A procedure is described for detecting unsaturation in the parent compound of colored derivatives at the microgram level using iodine monochloride. Included in the study were the 2,4-dinitrophenylhydrazone derivatives of carbonyl compounds, the 2,6-dinitrophenylhydrazone derivatives of pyruvic acid esters of alcohols, the  $\alpha$ -methyl-2,4,6-trinitrophenylhydrazone derivatives of alkoxyacetaldehyde compounds, the 2,6-dinitrophenylhydrazone derivatives of pyruvamide and the *N*-2,4-dinitrophenylethanolamine esters of fatty acids. Compounds which add iodine monochloride are sufficiently retarded on thin-layer partition plates to be classified as unsaturated. Carbonyl derivatives in which the double bond or bonds are conjugated with the hydrazone linkage and certain carbonyl compounds containing a double bond  $\beta,\gamma$  to the hydrazone linkage fail to be detected.

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