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PROBLEMS WITH ULTRAVIOLET SPECTROSCOPY OF FLAVONOIDS USING SODIUM ACETATE IN METHANOL

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Uv spectroscopy has been very useful in the structural identification of flavonoids (1). Comparison of the spectrum of the flavonoid in MeOH with spectra obtained by adding various reagents provides valuable information about the substitution pattern. One of these reagents, sodium acetate, is used to ionize only strongly acidic phenolic hydroxyls and is therefore useful for their detection. However, we report here studies indicating that the results with this reagent should be interpreted with caution.

The present study was prompted by observations in our laboratory which indicated that sodium acetate from different manufacturers yielded quite different spectra for the same flavonoid. It has been stated (1) that commercial-grade anhydrous sodium acetate contains varying amounts of HOAc; thus, one can expect varying pH values from different lots. These pH values are printed on the labels, but often pH changes occur with the aging of the sodium acetate. The pH values of various commercial sodium acetates we examined ranged from 6.7 to 8.5.

As a model flavonoid for spectroscopic experiments using different sources and preparations of sodium acetate, we chose 3,5,7,3',4'-pentahydroxyflavone (quercetin). Sodium acetate ionizes only the most acidic hydroxyls in flavonoids, notably the 3,7, and 4' hydroxyl group. However, the presence of 3' and 4' or 3,7, and 4' hydroxyl groups leads, respectively, to slow and fast decomposition, because these ionized systems readily interact with atmospheric oxygen. Depending on pH and impurities of the sodium acetate, we obtained spectra of quercetin showing a wide variety of shifts for the longwave band, ranging from almost zero to 74 nm. Furthermore, in some cases we observed instant decomposition, and in others the solutions were stable for more than 1 h.

Decomposition of quercetin (Table 1) is very fast above pH 8.0, while at pH 6.7 decomposition is slow and not complete even after 2 h. At pH 6.7, quercetin is not ionized, and the spectrum simply resembles the spectrum of quercetin in MeOH alone.

We also compared methods for preparing sodium acetate anhydrous and free of excess HOAc (as determined on the basis of pH measurements in a 5% aqueous solution), including oven-drying (30 min at 100°), lyophilization, and fusing. Fusing at the temperature of the melting point for 10 min always yielded the most basic products, giving solutions with pH-values of 10 or above. Lyophilization of a 20% frozen solution which had been brought to a pH of 6.5 with HOAc yielded a uniform, fine and anhydrous powder, producing solutions with a pH of 7.9-8.1. Oven drying of anhydrous sodium acetate for 30 min at 100° allowed preparation of solutions with pH from 6.83 to 8.22. Even air drying for 3 days removed much of the excess HOAc as evidenced by a solution pH increase from 6.83 to 7.85. When desiccation of anhydrous sodium acetate

TABLE 1. Uv Absorption Maxima of Quercetin in the Presence of Sodium Acetate

Brand	Lot #	pH on label	pH of sample	Absorption maxima[a]					Footnotes
				255	269 sh	301 sh	370		b
Baker	32133	6.8	6.8	**256**	274 sh	304 sh	329 sh	**379**	c
Baker	32133		7.2	**257**	272		329 sh	**380**	d
Baker	32133		7.8		275		**330**	*391*	e
Baker	32133		8.0		*275*	289 sh	**334**	391 sh	f
Baker	32133		8.2		*275*	289 sh	**330**	394 sh	g
Baker	36298	6.8	6.7	**257**	273 sh	300 sh	327 sh	**377**	c
Baker	36298		7.9		*275*		**333**	391 sh	h
Fisher	730567	8.9	8.4		*277*		**335**	398 sh	c
Fisher	730567		7.9	256 sh	*276*		**332**	398 sh	h
Baker	34119	8.0	8.3						i
Baker	34119		8.0	255 sh	**275**		**331**	**385**	h
Sigma	61C-3380		8.5						i
Sigma	61C-3380		7.7	258 sh	**274**		*325*	*388*	h
Sigma	61C-3380		8.1	258 sh	276		**334**	*390*	j
Fisher	790325	8.3	8.3		**274**		*331*	*412*	i
Fisher	790325		8.1		**276**		331 sh	**444**	h
Fisher	790325		8.3		**276**		*331*	**440**	j
Fisher	790325		6.4		**272**		330 sh	*433*	k
Aldrich (99.999%)	1150 DL		8.2	257 sh	276		**334**	392	

[a]Strongest absorption in bold, next in strength in italics.
[b]MeOH spectrum of quercetin.
[c]Sodium acetate from original container.
[d]Air-dried in hood for 24 h.
[e]Air-dried in hood for 72 h.
[f]Dried and kept under high vacuum and over KOH.
[g]Oven-dried at 100° for 30 min.
[h]Lyophilized at room temperature.
[i]Hydrous.
[j]Lyophilized at room temperature until dry, then at 85° for 20 h.
[k]Anhydrous, exposed to HOAc vapors.

under high vacuum over solid KOH was carried out for 72 h, the solution pH rose from 6.8 to 8.0.

The stability of anhydrous sodium acetate, regardless by which method it was prepared, depends on the number of times the container has been opened and how tightly it has been closed. In three of our lyophilized samples, we noticed pH increases of 0.1 to 0.3 pH units in a 5% aqueous solution after more than 6 months.

The key absorptions for quercetin are around 255, 275, 330, and 390 nm in sodium acetate. The 330 nm peak is derived from two absorptions, namely band III, which is an A-ring chromophore and a band associated with decomposition of the B-ring (this is important only in decomposing flavonoids). Band III is diagnostic for a free 7-hydroxyl group in non-decomposing flavones and flavonols (2). However, in decomposing compounds such as quercetin, Band III is quickly "swamped out" by the overlapping 330 nm absorption of "decomposed-quercetin". As decomposition proceeds, the most noticeable change is the disappearance of the peaks at both 390 and 255 nm and an increase in the absorptions at 330 and 275 nm. Eventually, the absorption at 275 nm also disappears. A totally decomposed quercetin will only produce a "decomposition peak" around 330 nm; this occurs above pH 8.2 within 10 min after the addition of sodium acetate.

A very special case was lot #790325 (see Table 1). After lyophilization, this sample of anhydrous sodium acetate in MeOH produced an extremely large bathochromic shift of quercetin's band I at 370 nm to 444 nm at pH 8.06. When the same lyophilized sodium acetate sample was subjected to a temperature of 90° for 20 h under high vacuum, the pH of the solution rose to 8.34. At both pH's quercetin was very stable, even after standing for an hour. In contrast, all other samples of sodium acetate lots caused rapid decomposition at any pH above 8. This stabilizing effect on a basic solution of quercetin may be explained by the presence of polyvalent ions. In order to find out the effects of these ions on the absorptions, we added the acetates of calcium and aluminum to the cuvettes. Only the aluminum ion produced a bathochromic shift to above 440 nm and a stable quercetin at pH's above 8. The following precautions are recommended for the use of sodium acetate for flavonoid spectroscopy:

When a new bottle of sodium acetate is opened, determine the pH of a 5% aqueous solution of the lot. If the pH is in the optimal range of 7.7-8.2, record a spectrum of quercetin in a saturated, methanolic solution of sodium acetate. The spectrum and, thus, the sodium acetate are acceptable if the absorbances fall into the following range: 255-258, 275-276 sh, 289 sh (this one may not always be perceptible), 330-335, and 385-394.

In cases where the pH is not in the range of 7.7-8.2, there is the option of either lyophilizing or replacing the material. One product[1] gave the best results. Whether lyophilized or fused sodium acetate is used, it is the pH of the prepared solution which is critical.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were recorded on a Gilford Ultraviolet-Visible Spectrophotomeer System 2600. The lyophilizations of sodium acetate were carried out in a Virtis Model No. 10-145 MR-BA instrument. The absence of crystal-water in the anhydrous sodium acetate was verified in a Perkin-Elmer DSC 4 Calorimeter.

LYOPHILIZATION.—A filtered, 20% solution (1 liter) of sodium acetate was frozen in a gauze-covered tray and lyophilized. This was usually carried out at ambient temperature without any shelf heat, except for two experiments in which we studied the effects of moderate heat (85°/20 h).

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[1]Aldrich Chemical Co., Gold label 99,999% (10 g=$16.00).

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