CHROMATOGRAPHIC SEPARATION OF THE DEGRADATION PRODUCTS OF PRALIDOXIME IODIDE (PYRIDINE-2-ALDOXIME METHIODIDE)

By Robert I. Ellin and David E. Easterday

From the Physiology Division, Directorate of Medical Research, U.S. Army Chemical Research and Development Laboratories, Army Chemical Center, Maryland

Received February 6, 1961

A satisfactory method for the separation and identification of *in vitro* breakdown products of pralidoxime iodide (PAM; pyridine-2-aldoxime methiodide; 2-hydroxyiminomethyl-*N*-methylpyridinium iodide) by paper chromatographic techniques is described.

Studies on the metabolic fate of 2-hydroxyiminomethyl-*N*-methylpyridinium iodide salts require suitable methods for analysing the *in vivo* degradation products. The present study of the chromatographic separation and identification of the *in vitro* breakdown products of pralidoxime iodide (PAM; pyridine-2-aldoxime methiodide; 2-hydroxyiminomethyl-*N* methylpyridinium iodide) represents a preliminary phase of the investigation.

Pralidoxime iodide breaks down *in vitro* to 2-formyl-*N*-methyl pyridinium iodide (PCAM; pyridine-2-aldehyde methiodide), 2-cyano-*N* methylpyridinium iodide (PCNM; 2-cyanopyridine methiodide), 2-hydroxy-*N*-methylpyridine (NMAP; *N*-methyl-α-pyridone), 2-carboxy-*N*-methylpyridinium iodide (PCOM; pyridine-2-carboxylic acid methiodide) and possibly 2-carbamyl-*N*-methylpyridinium iodide (PCMM; pyridine-2-carboxyamide methiodide) (Ellin, 1958).

In a recent study concerning the perfusion of ^{14}C—labelled pralidoxime iodide through liver, Way, Tong and Rabidean (1960) isolated a fraction which had chemical and spectral properties similar to those of PCNM. This investigation demonstrates that the aforementioned decomposition products can be qualitatively separated and identified by paper chromatographic methods. Furthermore, several distinct phenomena inherent in this experiment have been explained.

Experimental

All experiments were made with the descending technique on Whatman No. 1 paper chromatographic sheets, $18\frac{1}{4} \times 22\frac{1}{2}$ inches. A heavy walled Chromatocab (Research Equipment Corporation, Oakland, Calif.), was used for the chromatographic chamber. The samples were applied to the paper by 5 ml. self-filling pipettes and dried with a current of warm air. The paper was equilibrated for 4 hr. in the presence of both phases of the solvent system. Development required about 18 hr., in which time the solvent front travelled about 40 cm. After the sheets were air dried in a hood, the solvent front and the visible zones were marked under an ultra-violet lamp (Ultra-Violet Products, Inc., San Gabriel, Calif.). The sheets were then separately sprayed with the following reagents: (*a*) a

modified Dragendorff spray reagent (Schriftman and Kondritzer, 1957); (*b*) platinum spray reagent prepared by mixing 2 ml. of 10 per cent platinum chloride solution ($PtCl_4 \cdot 2HCl \cdot 6H_2O$) with 1 g. of potassium iodide in 98 ml. of water (Goldbaum and Kazyak, 1956); (*c*) an aqueous 1 per cent palladium chloride solution; (*d*) Tollen's spray reagent composed of two separate solutions: 0·1N silver nitrate, and 0·5N ammonia; (*e*) 0·4 per cent 2,4-dinitrophenylhydrazine reagent in 2N hydrochloric acid followed by 10 per cent aqueous potassium hydroxide. In routine practice it was convenient to run a series of paper strips of each compound and develop each strip with a different reagent.

The following solvent systems gave the best distribution and sharpest spots; (*a*) butanol: acetic acid: water (5 : 1 : 3), (*b*) water-saturated butanol; (*c*) 4 ml. acetic acid added to 100 ml. of butanol and the mixture saturated with water. Solvent systems were freshly prepared before use.

Results and Discussion

All compounds synthesised for the experiments were purified by recrystallisation or distillation. However, when developed with solvent system (*a*), each of the compounds tested, with the exception of NMAP,

TABLE I

R_F VALUES, AND TESTS TO DETECT PRALIDOXIME IODIDE AND ITS DEGRADATION PRODUCTS

Name	R_F*	Dragendorff reagent	Platinum reagent	Palladium reagent†	Ultra-violet light
Pralidoxime iodide	0·51	+	+	+	+
NMAP	0·81	−	−	−	+
PCNM	0·42	+	+	+	+
PCAM	0·62	−	−	+	+
PCOM	0·38	−	−	+	+
PCMM	0·37	+	+	+	+
Hydrogen iodide ..	0·20	−	−	+	−

* The listed R_F values are for a butanol: acetic acid: water system (5 : 1 : 3). To separate PCMM from PCOM, a 4 per cent acetic acid in butanol solvent saturated with water was used. Their R_F values were 0·08 and 0·15 respectively.

† The positive reaction is due to reaction with iodide ion.

produced two spots on the chromatogram (Fig. 1). One of the spots has an R_F value of 0·20, gives a positive reaction with palladium spray reagent and is not visible under the ultra-violet lamp. The other spots, with variable R_F values, were visible under the ultra-violet lamp but did not give positive reactions with palladium reagent. When developing solvent (*a*) was replaced by solvent system (*b*), a single spot was obtained for each compound. The single spots gave positive reactions with palladium reagent and were detected by ultra-violet light. The results were resolved when the spot which had a R_F value of 0·20 in solvent (*a*) was identified as hydrogen iodide. Solvent system (*a*) had effected separation of the iodide from the protonated organic bases, resulting in formation of the corresponding quaternary acetate and hydrogen iodide. Palladium reagent does not give a reaction with acetate ion. Thus, the positive reaction given by the various quaternary iodides with palladium spray can be attributed to reaction of the iodide ion and not the quaternary

moiety. This result was further confirmed by spotting pralidoxime nitrate (pyridine-2-aldoxime methonitrate) on chromatographic paper and developing with the acid solvent system. No spot giving a positive reaction with palladium spray reagent was found.

An unusual result occurred with PCAM. Before development with solvent system (*a*), the PCAM gave a positive reaction for aldehyde with both Tollen's and 2,4-dinitrophenylhydrazine reagent sprays. After development, the resulting spot gave a positive reaction only with 2,4-dinitrophenylhydrazine. This would indicate that the PCAM had reacted with

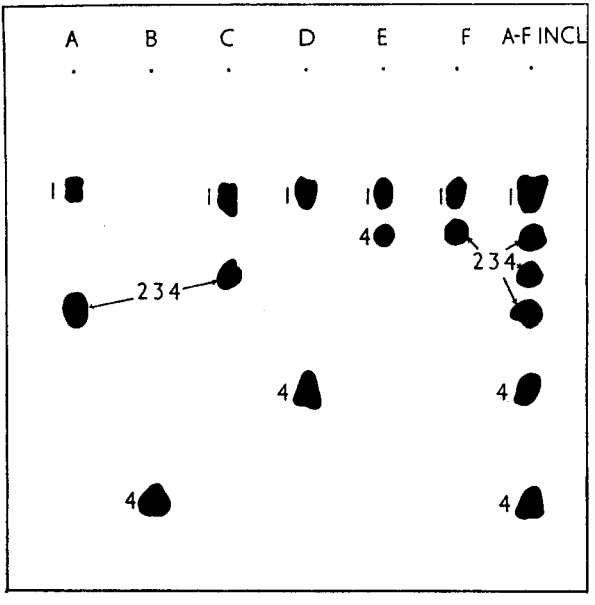

FIG. 1. Chromatogram of *in vitro* degradation products of pralidoxime iodide. A, Pralidoxime iodide; B, NMAP; C, PCNM; D, PCAM; E, PCOM; F, PCMM. A–F INCL, a mixture of the above compounds on a single spot. Positive reactions with: 1, palladium (ous)chloride spray; 2, Dragendorff spray; 3, platinum chloride spray; 4, ultra-violet light.

the alcohol in the solvent system to produce the corresponding hemiacetal or acetal.

In Table I are listed the various degradation products of pralidoxime iodide which have been studied. The R_F values given are the averages of 6 or more runs, using the descending method of development. The plus marks indicate those tests which are applicable for detecting the compounds. Variations in the R_F values of repeated runs were found to be less than ± 6 per cent.

When the five degradation compounds were spotted at the same point (Fig. 1, A–F INCL) on the chromatographic paper and developed with solvent system (*a*), a predictable separation was obtained. Two of the five compounds, PCOM and PCMM had similar R_F values and could not be separated by solvent system (*a*). The application of two dimensional

chromatography using a second solvent system, solvent (*c*), effected a satisfactory separation. The two spots were identified under ultraviolet light, the top spot being PCMM and the lower, PCOM.

Conclusions

Mixtures of *in vitro* degradation products of PAM have been separated by descending paper chromatography. A single solvent system can be used for the resolution of any combination of the compounds, with the exception of one. The latter required two-dimensional chromatography techniques. A number of spray reagents were used; reliance on the most sensitive reagent, palladium chloride, for detecting quaternary compounds would have led to erroneous conclusions. The spot corresponding to PCAM was actually the corresponding hemiacetal or acetal, but, nevertheless was satisfactory for the identification of the aldehyde component initially present.

References

Ellin, R. I. (1958). *J. Amer. chem. Soc.*, **80**, 6588–6590.
Goldbaum, L. R. and Kazyak, L. (1956). *Analyt. Chem.*, **28**, 1289–1290.
Kondritzer, A. A., Ellin, R. I. and Edberg, L. J. (1961). *J. pharm. Sci.*, **50**, 109–112.
Schriftman, H. and Kondritzer, A. A. (1957). *J. Amer. pharm. Ass., Sci. Ed.*, **46**, 173–176.
Way, J. L., Tong, H. and Rabideau, R. (1960). *Fed. Proc. Abstr.*, Part I, 19–276.