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SHORT COMMUNICATIONS

Effects of urushiol compounds isolated from poison ivy on ATPase activities

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In a recent article,* it was reported that a procedure for immunization of guinea pigs against allergic reactions to poison ivy had been developed. The chemicals causing the allergic response are called urushiols (3-C_{15} *n*-alkyl, catechol derivatives). Urushiols are also found in poison sumac and poison oak. The *n*-alkyl chain linked to a ring structure is somewhat similar to the piperdine derivatives (6-C_{11}, C_{13} and C_{15} *n*-alkyl) found in fire ant venom [1, 2].

Since the fire ant venom has been shown to be a strong inhibitor of ATPase activities [3, 4] and of oxidative phosphorylation [5], it was of interest to investigate the effects of the poison ivy urushiol components on these enzyme activities. Two urushiol components isolated from poison ivy were obtained as gifts from Dr. Sue Watson, RIAS, University of Mississippi: (1) PDC (3-*n*-pentadecyl catechol) and (2) Triolefinic (3 *n*-pentadecatrienyl catechol). The three double bonds in the Triolefinic component are located at C-8, -11, and -14. Dried samples as received were dissolved in 95% ethanol to yield solutions containing 8 mM PDC and 10.5 mM Triolefinic. Aliquots from these solutions were added to enzyme reaction mixtures using a microsyringe while rapidly stirring with a Vortex mixer. ATPase activities were measured according to the method of Pullman *et al.* [6] as described by Koch and Gilliland [7]. The urushiol components were added to the complete reaction mixture just before the addition of enzyme, followed 1 min later by final addition of ATP. Continuous measurement of changes in absorbance at 340 nm was begun 2–3 min later (after temperature equilibration at 37°).

Enzyme sources were tissue homogenate B-fractions nerve ending particle (NEP preparations according to Koch [8]) which were available (and well characterized) from other sources in the laboratory. Cow brain was the source of tissue for tests on Na^+–K^+ ATPase activity. The B-fraction used contained 8.7 μg protein/25 μl of sucrose solution, and the enzyme specific activity was 17.2 μmoles Pi·(mg protein)$^{-1}$·hr^{-1}. The source of tissue for measurement of mitochondrial Mg^{2+} ATPase activity was a B-fraction from a homogenate of boll weevil thorax. The specific activity of oligomycin-sensitive (OS)–Mg^{2+} ATPase was 15.5 μmoles Pi·(mg protein)$^{-1}$·hr^{-1} for a sample with a protein content of 23.4 μg/25 μl. Proteins were determined by the method of Lowry *et al.* [9], using bovine serum albumin (BSA) as the standard protein.

Figure 1 shows the effects of PDC† on ATPase activities from cow brain (Na^+–K^+) and the boll weevil (OS–Mg^{2+}) preparations. It is apparent that both enzyme activities were equally sensitive to PDC as seen by the nearly parallel lines and similar K_I values determined from the Dixon plots (Fig. 1). Similar responses were observed for low levels (<30 μM) of the Triolefinic† component (Fig. 2), and K_I values for enzyme activities from the two tissue preparations were in close agreement for the respective tissues compared to the PDC K_I values (Fig. 1). At concentrations above 30 μM Triolefinic, however, the inhibitor effects on the Na^+–K^+ and the OS–Mg^{2+} ATPase activities sharply diverged (Fig. 2). Although both ATPase activities showed greater relative sensitivity to the Triolefinic component, boll weevil OS–Mg^{2+} ATPase activity showed a much sharper increase in inhibition than Na^+–K^+ ATPase. No such increase in ATPase sensitivity was observed using the PDC component (Fig. 1).

The reason for the increase in ATPase sensitivity at higher Triolefinic concentrations is not immediately obvious. The presence of unsaturation in the *n*-alkyl side chain causes an increase in polarity and in the solubility of the Triolefinic component. The great increase in inhibition above about a 30 μM concentration (Fig. 2) could indicate the presence of low affinity binding sites on the enzyme molecules that were very sensitive at concentrations not attainable by the less soluble PDC component.

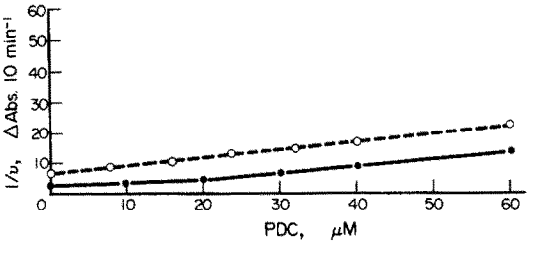

Fig. 1. Effect of the PDC component of poison ivy on cow brain Na^+–K^+ ATPase activity (○) and boll weevil thorax OS–Mg^{2+} ATPase activity (●). The K_I value for (○) was 34.5 μM and for (●), 42.0 μM. A 1-ml reaction mixture contained: 4.5 mM ATP, 5 mM $MgCl_2$, 100 mM NaCl, 20 mM KCl, 135 mM imidazole–Cl buffer (pH 7.5), 0.19 mM NADH, 0.5 mM PEP, 0.02% BSA, approximately 9 units pyruvate kinase and 12 units lactic dehydrogenase, and an appropriate aliquot of homogenatg fraction (see text). Absorbance changes were measured at 340 nm for 10 min using a Gilford 2400 recording spectrophotometer with temperature maintained at 37°. Total ATPase activity was determined in the presence of Na^+, K^+, and Mg^{2+}. Mg^{2+} ATPase activity was measured using the same mixture plus ouabain (1.0 mM). Na^+–K^+ ATPase activity is total activity minus Mg^{2+} ATPase activity. Oligomycin (2 μg/ml) was used to delineate oligomycin-sensitive (mitochondrial or OS-) and oligomycin-insensitive (OIS) Mg^{2+} ATPases. The data obtained were based on, for the most part, duplicate or more determinations. The variation in replicate values was less than 5 per cent of the average.

* *Chemical & Engineering News*, Aug. 27, 1979, by Drs. Watson, Elsohly and Waller, RIAS, School of Pharmacy, University of Mississippi, Oxford, MS, U.S.A.

† Stock solutions of PDC and Triolefinic were 8 and 10.6 mM, respectively, in 95% ethanol (1 μl/ml of reaction mixture equalled 8 and 10.6 μM final concentrations). Ethanol alone caused no observable inhibition of the ATPase activities at 5 μl/ml of reaction mixture. At 10 μl/ml, ethanol caused 5–10 per cent inhibition of Na^+–K^+ ATPase and less than 5 per cent of the Mg^{2+} ATPase activities.

In other unpublished investigations in this laboratory (R.B.K.), it has been observed that *n*-alkyl derivatives of primary amines and *n*-alkyl derivatives of nicotinamides have inhibitory effects on Na^+–K^+ ATPase activity obtained from dog brain NEP preparations. The length of the *n*-alkyl chain was important, the longer chains having greater inhibitory effects. A possible explanation for the above observations and for the effects of the urushiol components (Figs. 1 and 2) on ATPase activities may reside in their detergent-like structures, i.e. the polar end group and the non-polar hydrocarbon tail. The *n*-alkyl chain could be expected to enter the non-polar region of the lipoprotein complexes that constitute portions of the plasma membrane and are known to be the exclusive sites for Na^+–K^+ ATPase [10–13]. Similarly, the *n*-alkyl chain could enter the non-polar region of the inner mitochondrial membrane, the exclusive site of mitochondrial Mg^{2+} ATPase [6, 14, 15].

Ahmed and Thomas [16] found that Na^+–K^+ ATPase inhibition was dependent on chain length for a series of saturated fatty acids, and that mono-unsaturation increased inhibition by C_{16} and C_{18} fatty acids. Koch *et al.* [4], in studies on *n*-alkyl piperidines, proposed that *in vitro* inhibition of Na^+–K^+ ATPase from rat brain may result from interference with a conformation transition ($E_1P \rightarrow E_2P$) of the particulate (lipoprotein) enzyme fraction. Penetration of the non-polar chain into the non-polar regions of the lipoprotein complexes of the enzymes could permit interactions with otherwise inaccessible polar and/or charged groups that could have specific requirements for proper functioning of the enzymes. Such an interaction and/or disruption could be responsible for loss of enzyme activity.

The importance of the phospholipoprotein complex was indicated for odorant perturbation of Na^+–K^+ ATPase activity from olfactory tissue. Detergent (Lubrol WX) treatment of a membrane preparation from rabbit olfactory tissue caused a strong (56 per cent) inhibitory response of the enzyme activity at 10^{-3} M octanol [17]. In the absence of detergent treatment, 10^{-3} M octanol caused a 30 per cent stimulation of Na^+–K^+ ATPase activity from a rabbit olfactory tissue NEP preparation.

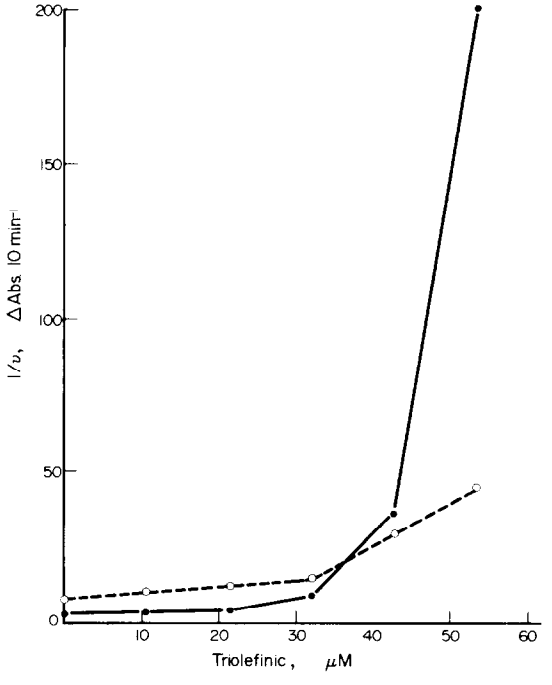

Fig. 2. Effect of the Triolefinic component of poison ivy on cow brain Na^+–K^+ ATPase activity (○) and boll weevil thorax OS-Mg^{2+} ATPase activity (●). The K_I value for (○) was 26.5 μM and for (●), 35 μM.

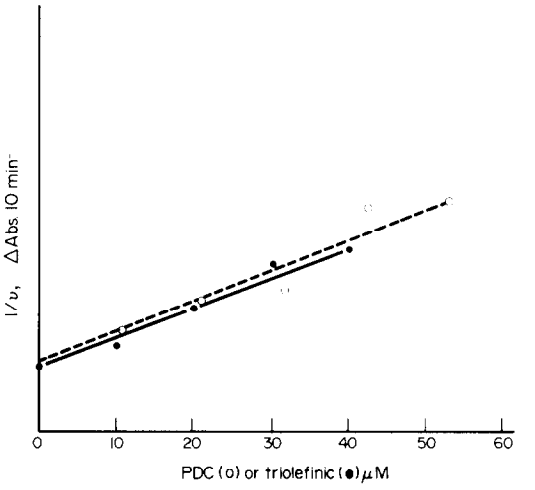

Fig. 3. Effects of PDC and Triolefinic components from poison ivy on boll weevil thorax OIS–Mg^{2+} ATPase activity. Key: (○) PDC and (●) Triolefinic components.

An evaluation of earlier studies [3] on ATPase inhibition by fire ant venom components, using Dixon plots, showed that the $C_{15:1}$ (*cis*-2-methyl-6-*cis*-6′-*n*-pentadecenyl) and C_{15} (*cis*-2-methyl-6-*n*-pentadecyl) piperidine derivatives caused inhibition response patterns similar to those of PDC and Triolefinic components. $C_{15:1}$ piperidine caused a sharp increase in the inhibition of OS–Mg^{2+} ATPase activity as observed with the Triolefinic component.

The two different types of toxic components (catechols and piperidines), however, showed quite different effects on oligomycin-insensitive (OIS)-ATPase activities. At 3.3×10^{-6} M $C_{15:1}$ and C_{15} piperidine, significant (*ca.* 40 per cent inhibition occurred. At 3.3×10^{-5} M the latter components (piperidines) caused stimulation (over 200 per cent) of OIS-ATPase activity, with $C_{15:1}$ being much more effective than the saturated C_{15}. This did not appear to be the case for urushiols. Triolefinic inhibition was greatest at 5.3×10^{-5} M, and no indication of reversal of inhibition of OIS-ATPase was observed (Fig. 3). Abd-El-Fattah [18] observed the same type of increased response of OIS–Mg^{2+} ATPase at 10^{-5} M with C_{15} and $C_{15:1}$ piperidine, when tested on a rat heart mitochondrial preparation. He proposed that the increase in OIS–Mg^{2+} ATPase activity was due to uncoupling of F_1-ATPase from the mitochondrial F_0–F_1 complex. Thus, although the C_{15}-catechol and piperidine derivatives have similar effects on Na^+–K^+ ATPase activity, they differ markedly in their effects on OS– and OIS–Mg^{2+} ATPase activities.

Use of the naturally occurring toxic compounds from poison ivy could prove helpful in understanding the role of the phospholipid requirement of membrane bound enzymes such as Na^+–K^+, and mitochondrial Mg^{2+} ATPase. Moreover, further studies should be conducted to determine whether the inhibitory action of these components of poison ivy and fire ant venom on the ATPase activities (and possibly other membranes bound enzymes) could have an association with their toxic actions.

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Studies on the mechanism of toxicity of metrizamide—competitive inhibition of yeast hexokinase

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Metrizamide, 2-[3-acetamido-2,4,6-triiodo-5-(*N*-methylacetamido)benzamido]-2-deoxy-D-glucopyranose (Fig. 1), derived from glucosamine and metrizoic acid, is useful as a density gradient medium [1–3] and as a radiologic contrast agent [4, 5]. These applications of metrizamide take advantage of its relatively low viscosity and high molecular weight (789.1), nearly half of which is due to the iodine in the metrizoic acid portion. Although metrizamide is considered an inert substance [1–3] and is relatively safe in comparison with previous contrast media [6–9], evidence of encephalopathy is found in many patients after its intrathecal use [10]. Intrathecally administered metrizamide is known to penetrate into the cerebral cortex [11], where it could affect metabolism within the cell. Although many of the toxic effects of radiological contrast agents have been attributed [12, 13] to the iodine containing moiety, we consider here the hypothesis that the glucose moiety of metrizamide may inhibit glycolysis. Accordingly, we have compared the effects of metrizamide on hexokinase (ATP: D-hexose-6-phosphotransferase; EC 2.7.1.1) to those of glucosamine, 2-deoxyglucose, and metrizoic acid. A preliminary report of this work has been presented [14].

Materials and Methods

Metrizamide, metrizoic acid, glucosamine, 2-deoxyglucose (grade III), glucose-6-phosphate and 2-deoxyglucose-6-phosphate were obtained from the Sigma Chemical Co. (St. Louis, MO), dextrose and urea from Matheson, Coleman & Bell (Norwood, OH), mannitol from Cutter Laboratories (Berkeley, CA), and glucose single vial reagent from CalBiochem-Behring (La Jolla, CA).

Reactions were carried out at 30° in semimicrocuvettes in a temperature regulated chamber of a Gilford model 250 UV-VIS spectrophotometer connected to an Esterline Angus model 575 X-Y plotter. When reconstituted in distilled water, the single vial reagent contained 50 mM Tris buffer (pH 7.6), 0.55 mM ATP, 7.4 mM $MgCl_2$, 0.59 mM NADP, 0.33 I.U. yeast hexokinase (EC 2.7.7.1), and 0.16 I.U. yeast glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate phosphohydrolase; EC 3.1.3.9). The reactions were begun by the addition of 10 μl of 1, 2, 3, 4, 6 or 8 mM glucose in 0, 20, 50 or 100 mM metrizamide (or 2-deoxyglucose or glucosamine) to 500 μl of single vial reagent. After the cuvettes were inverted quickly twice,