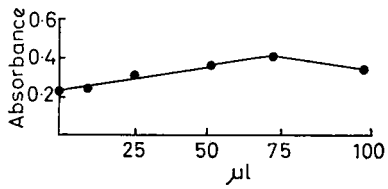

FIG. 2. Effect of adding water (1 µl) on the absorbance of pilocarpine hydrochloride/MAA complex.

or 0·002% benzalkonium chloride (pilocarpine hydrochloride), a suitable volume equivalent to 2–3 mg of the amine was pretreated by adding 1 ml of acetic anhydride before heating with the MAA reagent. Water affects the absorbance of the condensation product, but in a reproducible manner (Fig. 2) and provided the amount of water in the calibration standard and assay sample is the same, accurate results may be obtained (Table 2).

Typically, 50 µl aliquots of pilocarpine hydrochloride eye drops were taken and 50 µl of 0·002% benzalkonium chloride solution was added to the pilocarpine hydrochloride standard before reaction. Alternatively the solvent can be evaporated and the MAA reagent added to the dry residue. If the amine is present in a complex mixture such as a linctus, or is in tablet form, preliminary extraction of the amine is necessary. Noscapine hydrochloride was extracted by the method of the British Pharmaceutical Codex (1968).

Table 3. *Comparison of limits of detection for MAA/-spectrophotometric method with some other spectrophotometric methods.*

	Literature value (µg ml^{-1})	This study (µg ml^{-1})
Ampicillin trihydrate	2[a]	0·28
Pilocarpine hydrochloride	0·1[b]	0·02
Promethazine hydrochloride	60[c]	0·03

a. Celletti, Moretti & Petrangeli (1972).
b. Repta & Higuchi (1971).
c. Tarasiewicz (1972).

The method presented here compares well with other spectrophotometric methods of analysis for the various amines in terms of the speed of the reaction and the limits of detection (Table 3). A lowering of the limit of detection by at least an order of magnitude is possible if a spectrofluorometric method of analysis is used.

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REFERENCES

British Pharmaceutical Codex (1968). p. 1133. London: The Pharmaceutical Press.

CELLETTI, P., MORETTI, G. P. & PETRANGELI, B. (1972). *Farmaco, Ed. prat.*, **27**, 688–698; *Analyt. Abs.* (1973), **25**, 427.

FEIGL, F. (1966). *Spot Tests in Organic Analysis, p.* 251, 7th Ed. Amsterdam: Elsevier.

PERRIN, D. D. (1965). *Dissociation Constants of Organic Bases in Aqueous Solvents* plus Supplement 1972. London Butterworth.

REPTA, A. & HIGUCHI, T. (1971). *J. pharm. Sci.*, **60**, 1465–1470.

ROEDER, G. (1941). *J. Am. pharm. Assoc.*, **30**, 74–76.

TARASIEWICZ, M. (1972). *Acta Pol. pharm.*, **29**, 583–589; *Analyt. Abs.* (1973), **25**, 440.

THOMAS, A. D. (1975). *Talanta*, **22**, 865–869.

WILSON, A. L. (1961). *Analyst*, **86**, 72–74.

Anti-inflammatory activity of esters of acetic acid

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Phenylglycine n-heptyl ester has been found in both *in vitro* and *in vivo* tests to be a potent inhibitor of bradykinin, 5-hydroxytryptamine (5-HT), histamine and dextran in rats and of acetylcholine, histamine, 5-HT and anaphylaxis in guinea-pigs (Gecse, Zsilinszky & others, 1971). Recently, this compound and the corresponding ester of phenylalanine were shown to inhibit carrageenan and dextran responses in rat paws as well as arthritis induced by Freund's adjuvant in rats (Thomas & West, 1973). More recently, using a series of straight-chain esters of phenylglycine, Thomas & West (1974) reported that anti-inflammatory activity was possessed only by esters of phenylglycine with high molecular weight alcohols, the threshold being hexyl for dextran oedema and pentyl for carrageenan inflammation. Optimal activity in both types of inflammation resided in the heptyl ester.

We have now tested a series of straight-chain esters of acetic acid for their anti-inflammatory effects. Inflammation was induced by subcutaneous injections of carrageenan (1 mg in 0·1 ml normal saline) into the right hind-paws of groups of 6 Wistar rats (120–150 g) obtained from the Tuck colony. The increase in paw volume was recorded every hour for 5 h on a volume

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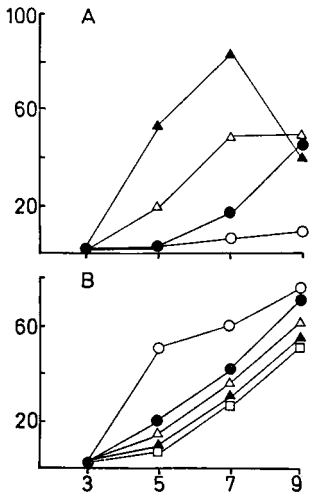

FIG. 1. Relation of alcohol chain length in esters of acetic acid (abscissa) and activity against (A) carrageenan oedema in rat paws measured as mean percentage inhibitions at 3 h (ordinate). Doses used were 50 (○), 67 (●), 100 (△) and 200 (▲) mg kg^{-1} intraperitoneally. Note the peak of activity with a 7 carbon chain length at the higher doses; and
(B) Bradykinin (100 ng, ○), prostaglandin E_2 (100 ng, ●), 5-HT (100 ng, △), histamine (100 μg, ▲) and dextran (500 μg, □) injected intradermally into rat skin (ordinate). Dose of ester was 100 mg kg^{-1} intraperitoneally and dye exudate, measured on an arbitary scale, is recorded as mean percentage inhibition of control responses.

differential meter. Esters (dissolved in 1% Tween 80) were administered intraperitoneally 30 min before the inflammatory stimulus. The percentage inhibition of inflammation was calculated by comparing the mean percentage increase in the paw volume of the control group with the mean percentage increase in the paw volume of the treated group.

Fig. 1A shows the results obtained after 3 h using 4 dose levels of the esters. Maximum inhibition of the carrageenan response occurs with the heptyl compound at 200 mg kg^{-1}. This inhibition is comparable with that found by Thomas & West (1974) for the heptyl ester of phenylglycine at 100 mg kg^{-1}, so that heptyl acetate is about half as active. With pentyl acetate at 100 mg kg^{-1}, the inhibition of nearly 22% is comparable with that found previously for the pentyl ester of phenylglycine. As with the series of phenylglycine esters, the propyl compound is inactive at doses up to 200 mg kg^{-1}.

The effect of these esters of acetic acid was also investigated on the extravasation of azovan blue dye following intradermal injections of different putative mediators of the inflammatory response into the shaved dorsal skin of groups of 6 rats. Test compounds were given intraperitoneally in 1% Tween 80 30 min before the phlogistic agent and azovan blue dye was given intravenously at a dose of 30 mg kg^{-1}. Thirty min later, the animals were killed, the pelts removed, and the intensity of the dye visually assessed on an arbitary scale. The mean percentage inhibitions of the dye extravastion by the test compounds using 100 mg kg^{-1} are shown in Fig. 1B. The esters with the 3 higher molecular alcohols were most active against bradykinin, less active against PGE_2 and 5-HT, and least active against histamine and dextran. This order of antagonism for the heptyl ester of acetic acid is in line with that reported by Gecse & others (1971) for the heptyl ester of phenylglycine.

These results show that, by increasing the chain length of the alcohol in the ester linkage of acetic acid, the inhibition of the carrageenan response in rat paws is enhanced. However, there is a limit to the enhancement for (*a*) doubling the dose of the nonyl ester does not result in an increase in activity, and (*b*) the heptyl ester at 100 mg kg^{-1} is no more active than the nonyl ester at the same dose. Thus, it is possible that the inhibitory site for the esters is too small for the nonyl compound and may be optimally filled by the heptyl compound provided there is a sufficient concentration achieved at the inflammatory site. Such a hypothesis is not in line with the findings of Thomas & West (1974) who found that the nonyl ester of phenylglycine was equally active with the heptyl ester in antagonising both carrageenan and dextran responses in rat paws.

The experiments with rat skin clearly show that the inhibitory activity against the putative mediators bradykinin, histamine, 5-HT and prostaglandin PGE_2, also increases with alcohol chain length, even in the present series of esters of acetic acid, and this may account for the results obtained in the carrageenan study. The importance of the ester bond has been established as each alcohol used, as well as acetic acid, were inactive in these tests.

Finally, none of the acetate esters was found to be active orally in doses up to 200 mg kg^{-1}.

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REFERENCES

GECSE, A., ZSILINSZKY, E., LONOVICS, J. & WEST, G. B. (1971). *Int. Archs Allergy*, **41**, 174–179.
THOMAS, G. & WEST, G. B. (1973). *Br. J. Pharmac.*, **47**, 662P.
THOMAS, G. & WEST, G. B. (1974). *J. Pharm. Pharmac.*, **26**, 151–152.