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1. Schlittler, E. and Bein, H. J., In *Antihypertensive Agents*, E. Schlittler, ed., Academic Press, New York, 1967, p. 191.
2. Erspamer, V. and Asero, B., *Nature (London)*, 1952, **169**, 800.
3. Shaw, E. and Wooley, Q. W., *J. Pharmacol. Exptl. Therap.*, 1954, **111**, 439.
4. Arnold, A., Selberies, W. H., *Experientia*, 1967, **23**, 298.
5. Hukner, Charles, F., *Ger. Ofien*, 2, 609, 289 (Cl. 007 2401/14) 30 Sept. (1976)- *U.S. Appl.* 556, 600, 10 March, 1975, 72 pp.
6. Archibold, John, L., Takson, John Lambert, *Brit.*, 1, 413, 708 (Cl. 007D, 461K) Nov. (1975), *Appl.*, 29. 156/73, 19 June (1973), 2 pp, Add. to *Brit.*, 1, 218, 570.
7. Alps, B. J., Archibald, J. L., Johnson, E. S. and Wilson, A. B., *Cardiovasc. Res.*, 1970, p. 62.
8. Alps, B. J., Tull, N, Johnson, E. S. and Wilson, A. B., *Ibid.*, 1970, **40**, 153.
9. Rapport, M. M., Green, A. A., Page, J. H., *Science*, 1948, **108**, 329.
10. Rapport, M. M., *J. Biol. Chem.*, 1949, **180**, 961.
11. Hamlin, K. E. and Fisher, F. E., *J. Am. Chem. Soc.*, 1951, **73**, 5007.
12. Allen, C. F. H. and VauAllan, *J. Org. Syn. Coll.*, 1955, **3**, 597.
13. Vogel, I., *A Textbook of Practical Organic Chemistry*, 1971, **851**, 852.
14. Albro, L. P., Baltzly, R. and Phillips, A. P., *J. Org. Chem.*, 1949, **74**, 771.
15. Jerry, A., Weisbach, Edward, Macko, Nicholas, J. De, Sanctis Michael, P., Cacava and Bryce Douglas, *J. Med. Chem.*, 1964, **7**, 738.
16. Dubey, M., Verma, V. K., Shanker, K., Sinha, U. N., Bhargava, K. P. and Kishor, K., *Pharmazie*, 1978, **33** (10), 640.
17. Smith, C. C., *J. Pharmacol. Exp. Ther.*, 1943, **77**, 392.

ESTIMATION OF RARE EARTH COMPLEXES OF GLUTAMIC ACID WITH CHLORAMINE-T AND CHLORAMINE-B

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ABSTRACT

Chloramine-T(CAT) and Chloramine-B(CAB) have received considerable attention as oxidimetric reagents[1–4]. The mono sodium salt of glutamic acid finds extensive commercial use as a flavour intensifier, while the acid itself is used in medicine and biochemical research, and as a salt substitute and dietary supplement. Rapid methods have been developed for the estimation of this amino acid[5,6] in solution with CAT and CAB. In view of the importance of the metal protein complexes in organic chemistry, a study of metal-amino acid complexes is worthwhile as these are helpful in the separation and identification of amino acids. The present communication reports an elegant method for the estimation of trivalent rare earth complexes of glutamic acid with CAT and CAB.

MATERIALS AND METHODS

THE rare earth complexes of glutamic acid having the general formula $M(glut\text{–}H)_3 \cdot 3H_2O$ where M is Y, La, Pr Nd, Sm, Gd, Tb, Dy and Ho were prepared by mixing stoichiometric amounts of amino acid and the rare earth carbonate in aqueous solution[7]. The composition was checked by determining the rare earth content by the oxalate method[8]. Chloramine-T was purified by the method of Morris *et al.*[9]. Chloramine-B was prepared[10] by passing chlorine through benzene sulphonamide dissolved in 4N NaOH solution over a period of one hour at 70°. The mass obtained was filtered, dried and crystallised from water. Approximately decinormal aqueous solutions of CAT and CAB were prepared and standardised by the iodometric method.

Preliminary Studies

Oxidation studies were carried out at different pH but the reaction was stoichiometric and rapid at pH 4, hence further studies were carried out at this pH. Known amounts of complex solution (~2 mg/ml) in acetate buffer of pH 4 were added to a known excess volume of CAT or CAB solution (~ 1·25 m mole) in an iodine flask at 25° (± 2°). The reaction mixture was set aside for various intervals of time with occasional shaking. The excess of oxidant was then determined by iodometric titration and the time required for the stoichiometric oxidation of the complex was noted. It was found that oxidation is complete within five minutes, with a 12-electron change per complex molecule (Table I).

TABLE I

Extent of oxidation of rare earth complexes of glutamic acid with chloramine-T and Chloramine-B at pH 4 buffer medium

Rare earth complex	mole of CAT consumed	mole of CAB consumed
	mole of complex taken	mole of complex taken
$Y(C_5H_8O_4N)_3 \cdot 3H_2O$	5·99	6·01
$La(C_5H_8O_4N)_3 \cdot 3H_2O$	6·02	6·03
$Pr(C_5H_8O_4N)_3 \cdot 3H_2O$	6·01	6·02
$Nd(C_5H_8O_4N)_3 \cdot 3H_2O$	6·02	6·03
$Sm(C_5H_8O_4N)_3 \cdot 3H_2O$	6·01	6·00
$Gd(C_5H_8O_4N)_3 \cdot 3H_2O$	5·99	5·99
$Tb(C_5H_8O_4N)_3 \cdot 3H_2O$	5·99	6·01
$Dy(C_5H_8O_4N)_3 \cdot 3H_2O$	5·99	5·99
$Ho(C_5H_8O_4N)_3 \cdot 3H_2O$	5·99	6·00

Complex taken : 0·032 m mole
Oxidant taken : 1·25 m mole
Time : 5 min.
Temp. : 25 ± 2° C

Recommended Procedure

Aliquot portions of the solution of the complex (~4–40 mg) in acetate buffer of pH 4 were added to 25 ml of 0·1N CAT or CAB solution in an iodine flask at room temperature. The mixture was shaken thoroughly and after five minutes, 10 ml of 2N H_2SO_4 and 20 ml of 20% KI solution were added and the liberated iodine titrated against standard thiosulphate using starch indicator. A blank titration was carried out with the same volume of CAT or CAB solution alone.

Results and Discussion

Determination with 4–40 mg of the complex were achieved in acetate buffer with a maximum error of 1·0%. Typical results are given in Table II.

TABLE II

Estimation of rare earth complexes of glutamic acid with chloramine-T and chloramine-B

Rare earth complex	Range studied (mg)	Error %	
		CAT	CAB
$Y(C_5H_8O_4N)_3 \cdot 3H_2O$	3–30	0 0·72	0–0·68
$La(C_5H_8O_4N)_3 \cdot 3H_2O$	4–40	0–1·00	0–0·77
$Pr(C_5H_8O_4N)_3 \cdot 3H_2O$	4–40	0–0·77	0–0·77
$Nd(C_5H_8O_4N)_3 \cdot 3H_2O$	4–40	0–1·00	0–0·89
$Sm(C_5H_8O_4N)_3 \cdot 3H_2O$	4–40	0–1·00	0–0·89
$Gd(C_5H_8O_4N)_3 \cdot 3H_2O$	4–40	0–0·50	0–0·50
$Tb(C_5H_8O_4N)_3 \cdot 3H_2O$	4–40	0–0·50	0–0·98
$Dy(C_5H_8O_4N)_3 \cdot 3H_2O$	4–40	0–0·24	0–0·50
$Ho(C_5H_8O_4N)_3 \cdot 3H_2O$	4–40	0–0·40	0–0·50

p-Toluene sulphonamide and benzene sulphonamide among the reaction products were detected by paper chromatography[5] and TLC technique[6] respectively. The presence of nitrile in the reaction product was detected by its colour reaction with hydroxylamine and ferric chloride[11].

The stoichiometry is unaffected by the order of addition of oxidant and the complex.

Many commercial pharmaceutical preparations contain glutamic acid as a major component along with riboflavin and nicotinamide. The latter do not interfere in the estimation (upto 25 mg).

Ions such as Ba^{2+}, K^+, Zn^{2+} NO_3^-, PO_4^{3-}, SO_4^{2-}, ClO_4^- and urea had no effect while folic acid (1·7 mg), glycine (1·0 mg), methionine (1·0 mg and thiourea (1·0 mg) interfere.

It can be concluded that the number of ligand molecules present in a molecule of the rare earth complex could easily be computed by oxidation with CAT or CAB. The proposed analytical technique is rapid and accurate and is useful for estimating substantial amounts of these complexes by a proper adjustment of the reaction conditions.

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1. Aravamudan, G. and Venkappayya, D., *Talanta*, 1968, **15**, 704.
2. Mahadevappa, D. S. and Naidu, H. M. K., *Ibid.*, 1973, **20**, 349.
3. Srivatsva, A. and Bose, S., *J. Indian Chem. Soc.*, 1975, **52**, 214.
4. Yathirajan, H.S., Rangaswamy and Mahadevappa, D. S, *Ibid.*, 1979, **56**, 421.
5. Katgeri, S. N., Mahadevappa, D. S. and Naidu, H. M. K, *Talanta*, 1979, **26**, 420.
6. Rangaswamy, Yathirajan, H. S. and Mahadevappa, D. S., *Indian J Chem.* (in press).
7. Farooq, O. and Ahmad, N., *Acta Chim.* (Budapest), 1975, **85**, 395.
8. Barratt, M F., Mc Donald, T. R. R. and Topp, N. E., *J. Inorg. Nucl. Chem.*, 1964, **26**, 931.
9. Morris, J. C., Salazar, J. A. and Winemann, M. A., *J. Am. Chem. Soc.*, 1948, **70**, 2036.
10. Chrzaszczewska, A., *Chem. Abstr.*, 1955, **49**, 212.
11. Soloway, S. and Lipschietz, A., *Analyt. Chem.*, 1952, **13**, 976.

SEX-SPECIFIC DIFFERENCES IN TOTAL ESTERASE, ACETYLCHOLINE AND ACETYLCHOLINESTERASE OF HEADS OF *PHILOSAMIA RICINI* ADULT MOTHS

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ABSTRACT

Some sex-specific differences with regard to the activity of esterases (total and acetylcholine) and acetylcholine content have been studied in the head part of *Philosamia ricini* virgin moths. The cholinergic system of females seems to be relatively more active than that of males.

INTRODUCTION

ACETYLCHOLINE is present in high concentrations in insects when compared with most other invertebrates and vertebrates. In vertebrates, it plays a vital role in preganglionic transmission of the autonomic nervous system, at the nerve endings of the parasympathetic system, skeletal neuromuscular junctions[1–3] and in the brain[4]. The distribution of acetylcholinesterase, whose role in nerve transmission has been established, has been sought by biochemical and histochemical techniques. Histochemical results show that acetylcholinesterase occurs in the nervous tissue, muscle, digestive organs, reproductive organs and haemolymph of *Periplaneta*[5,6]. Although the presence of acetylcholine, acetylcholinesterase and cholineacetylase has been detected in many insects[7], the highest activity has been demonstrated in the most active insects such as *Musca, Apis* and *Periplaneta*[8]. In addition to the basic requirement for normal functioning of the nervous system, some insects may need more acetylcholine necessary for more specialized purposes[9].

The present study was undertaken to see if any sex-specific differences occurred in the lepidopteran, *Philosamia ricini*.

MATERIALS AND METHODS

Virgin male and female moths were sorted immediately after emergence. Moths of known age were chilled briefly in a refrigerator and their heads clipped off. A 1% (w/v) homogenate each of the male and female heads during adult development was prepared in glass-distilled water, employing 10 heads in each case.

All assays were made in duplicate sets on two individual homogenates and the mean values have been calculated. Acetylcholine was assayed by the method of Hestrin[10] as described by Metcalf[11] with modification. Esterases (total and acetylcholin-) were assayed by the method of Ellman *et al.*[12]. The method involves the colorimetric determination of sulfhydryl groups produced by enzymic hydrolysis of acetylthiocholine. The reaction mixture consisting of sodium phosphate (3·5 ml, 0·1 M, pH 8·0), colour reagent [0·2 ml, 0·0063 M 5·5′-dithiobis-2-nitrobenzoic acid (Sigma Chemical Company) in 0·065 M sodium phosphate (pH 7·0) containing sodium bicarbonate, 1 mg/ml], substrate (0·2 ml, 0·0095 M acetylthiocholine iodide, stability 3 days) and tissue homogenate (0·6 ml, 1% w/v) was incubated both in presence and absence of eserine solution (0·01 ml, 1·1 mg/ml) for 30 minutse