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COMMUNICATIONS

Possible Role for Trichloroacetate in Pharmaceutical Formulations

Keyphrases ☐ Trichloroacetate role—pharmaceutical formulations ☐ Thiopental sleeping time—trichloroacetate effect ☐ Protein binding, thiopental—trichloroacetate effect

Sir:

Trichloroacetic acid (TCA) has a pKa of 0.70 at 25° (1) and our investigations into its absorption from the rat's stomach using isotonic TCA buffers of pH 2.0 and pH 3.0 (2, 3) suggest it to be mainly absorbed as the undissociated acid. These experiments were performed to determine whether or not dextromethorphan (DMX) and tetracycline were absorbed in association with TCA (that is as ion pairs) following our earlier observations that DMX (4) was absorbed as a protonated species from chloride buffers. The investigations suggested that the drugs were not absorbed as ion pairs, and in the case of DMX the enhanced absorption from the TCA buffers seemed to be due to the increased surface activity of the drug. In a recent publication (5) the enhanced biological activity of a quaternary ammonium compound (isopropamide iodide) administered orally with a large excess of TCA has been reported; however, it is possible that the increased biological activity reported is not due to increased absorption but due to a biological availability phenomenon such as protein binding. TCA, a common protein precipitant, is strongly bound to serum proteins, and if, as suggested above, TCA is absorbed as the undissociated species from the upper alimentary tract, then this TCA could prevent or minimize the binding of the drug to the plasma proteins or tissues, thus freeing the drug for absorption at the biophase.

To check this possibility, we determined the LD_{50} and the sleeping time of mice after the intravenous injection of thiopental in solutions made isotonic with sodium chloride or trichloroacetic acid with the pH adjusted to 7.4. Thiopental (Pentothal sodium, Abbott Laboratories, North Chicago, Ill.) was used so that no ion-pair phenomenon could account for the results. It is very strongly protein bound (6, 7), and the biological responses are comparatively easy to evaluate. The drug in 0.2-ml. doses was injected into the tail vein of a 20-g. mouse (male, albino, Rolfsmeyer, Madison, Wis.); the duration of anaesthesia was determined by the righting reflex, the mouse being required to right itself three times in a period of 30 sec.

Table I—Sleeping Times

Thiopental Dose Level, mg./kg.	Trichloroacetate	Chloride
20	3.9 min., 3.5–4.8	2.2 min., 1.8–2.5
25	6.2 min., 4.8–9.0	3.4 min., 2.0–4.3
30	11.5 min., 6.5–22.0	4.1 min., 2.8–6.5

Using six animals at each dose level, the LD_{50} of thiopental in the TCA solution was 41.0 mg./kg. and in the sodium chloride solution was 61.0 mg./kg. The sleeping times for several dose levels using six animals at each dose level are summarized in the table. Animals injected with the isotonic solutions alone showed no untoward effects either immediately or during a period of 2 weeks (see Table I). Using the Student t test of significance ($p = 0.01$) the duration of sleeping times at each dose level was significantly different for the two solutions. In preliminary dialysis experiments using 50 mcg./ml. thiopental and 1% human albumin crystalline 100% (Mann Research Labs., New York, N. Y.), it was found that 55.0% of the thiopental was bound using physiological phosphate buffer whereas only 21.6% thio-

pental was bound when a sixteen-fold molar excess of TCA (a similar excess to that used in the *in vivo* experiments) was incorporated into the buffer. This suggests a competitive protein binding phenomenon that may account for the above differences, and pharmacologically inert but highly protein bound materials such as TCA may have a role in pharmaceutical formulations, particularly those parenterally administered.

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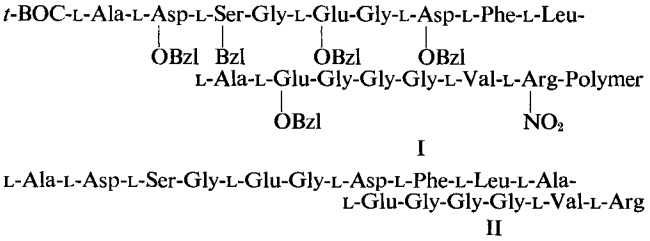
Rapid Peptide Synthesis: Synthesis of Human Fibrinopeptide A

Keyphrases ☐ Fibrinopeptide A, human—synthesis ☐ Column chromatography—separation ☐ Optical rotation—identity ☐ Paper chromatography—purity testing ☐ Electrophoresis—purity testing

Sir:

Recently the *N-t*-butyloxycarbonyl-L-amino acid pentachlorophenyl esters have been described (1) and shown to be useful intermediates for extremely rapid peptide synthesis (2). We wish to report a further application of these intermediates for use with the solid phase or Merrifield method (3) of peptide synthesis. For this purpose the first synthesis of the naturally occurring human peptide, fibrinopeptide A, (4, 5) L-alanyl-L-aspartyl-L-serylglycyl-L-glutamyl-glycyl-L-aspartyl-L-phenylalanyl-L-leucyl-L-alanyl-L-glutamyl-glycylglycylglycyl-L-valyl-L-arginine II, is described The synthesis is shown in Scheme I, its facility being due to the ease of purification of the starting amino acid pentachlorophenyl esters (1) and their high activity towards aminolysis (6).

The fully protected, resin bound, hexadecapeptide, fibrinopeptide A (I), was synthesized by the stepwise addition of the appropriate *N-t*-butyloxycarbonyl-L-amino acid pentachlorophenyl ester to an insoluble polystyrene resin (7) substituted with 0.43 mmoles/g.



Scheme I

of *N-t*-butyloxycarbonyl-nitro-L-arginine. The cycle for the addition of each amino acid residue consisted of the following steps; removal of the *N-t*-butyloxycarbonyl protecting group by treatment with excess 1 *N* hydrogen chloride in glacial acetic acid for 30 min.; neutralization of the resulting hydrochloride salt with excess triethylamine in dimethylformamide, and then coupling the resulting free amino residue with three equivalents of the appropriate *N-t*-butyloxycarbonyl-L-amino acid pentachlorophenyl ester in dimethylformamide for 12 hr. At the end of this chain-lengthening sequence, the protected peptide was cleaved from its polymer support by hydrogen bromide in trifluoroacetic acid, under which conditions the side-chain protecting groups, except that on arginine, were also cleaved. The partially protected peptide was then catalytically hydrogenated over 10% palladium on charcoal to give the crude hexadecapeptide, fibrinopeptide A. The peptide was purified by passage through a column of synthetic polysaccharide (Sephadex G-25) (100 × 2.5 cm.), using water as eluent at a flow rate of 6 ml./hr. The pure fibrinopeptide A, (II) $[\alpha]_D^{28}$ −44.8° (c 2.30 in water), was eluted as the first major fraction (13% overall yield) and the amino acid ratios[1] were: Ala, 2.0; Arg, 0.9; Asp, 1.9; Glu, 2.2; Gly, 5.3; Leu, 1.1; Phe, 1.0; Ser, 0.9; Val, 0.8. It was found to be homogeneous by paper chromatography; R_f 0.75 (butanol–acetic acid–water, 2:2:1); R_f 0.86 (phenol saturated with water). Electrophoresis on paper gave a single spot in phosphate buffer pH 6.9, which migrated 3.5 cm. towards the anode at 10 v/cm. for 2 hr.[2].

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