Gas Chromatographic Determination of *N*-γ-Phenylpropyl-*N*-benzyloxy Acetamide (W-1372) in Blood

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Abstract □ A procedure is described for the determination of *N*-γ-phenylpropyl-*N*-benzyloxy acetamide (W-1372) in plasma or whole blood. W-1372 is extracted from plasma with hexane and measured quantitatively by gas chromatography. The technique is simple, reproducible, and accurate in the range of 1–10 mcg./ml. Dibutyl phthalate is used as the internal standard for quantitation by the relative peak area method.

Keyphrases □ *N*-γ-Phenylpropyl-*N*-benzyloxy acetamide (W-1372)—determination, blood □ Blood—W-1372 drug determination □ GLC—analysis

W-1372 is a new hypolipidemic agent which has been shown by Berger *et al.* (1, 2) to retard atherosclerotic lesions and reduce blood cholesterol in animals maintained on a hypercholesteremic diet. This manuscript describes a procedure for the quantitative determination of W-1372 in blood.

EXPERIMENTAL

Equipment and Reagents—An F and M model 402 dual-column gas chromatograph, equipped with a hydrogen-flame ionization detector and a 1-mv. Minneapolis-Honeywell recorder, was employed. The chromatographic columns used were 1.23-m. × 0.63-cm. (4-ft. × 0.25-in.) glass tubes packed with 3.8% methylvinyl silicone gum rubber (W98) on 80–100 mesh Diataport S. The temperatures used were: column, 200°; injection port, 275°; and detector block, 225°. Carrier gas (helium) flow was 60 ml./min.

Sensitivity settings were range 10 with an attenuation factor of 4×. The reagents were redistilled hexane, redistilled chloroform, and dibutyl phthalate (Eastman). The retention times under these conditions are 4.8 min. for W-1372 and 1.6 min. for dibutyl phthalate (Fig. 1).

Procedure—Plasma or whole blood, 1.0 ml., was extracted with 10 ml. of hexane, and 8.0 ml. of the organic solvent was removed and evaporated to dryness in a stream of nitrogen. For drug concentration greater than 10 mcg./ml., the specimen was diluted with physiological saline to an appropriate volume prior to extraction. The dried sample was dissolved in 0.1 ml. of chloroform containing 1.0 mcg. of dibutyl phthalate, and 2.6 μl. of this solution was injected into the gas chromatograph. The concentration of W-1372

Table I—Recovery of W-1372 Added to Human Plasma

mcg./ml. Added	Recovery, %[a]
2.0	99.2 ± 1.0
5.0	98.0 ± 1.1
10.0	98.3 ± 0.6

[a] Values are the mean of quadruplicate determinations and are given with their standard error.

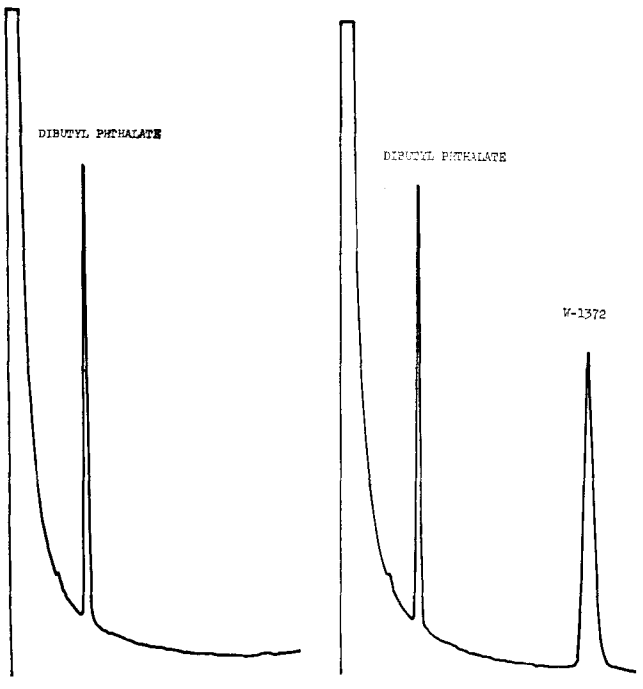

Figure 1—*Gas chromatograms of human plasma treated as described. Left: Chromatogram from normal plasma. Right: Chromatogram from normal plasma with 10 mcg./ml. of W-1372 added.*

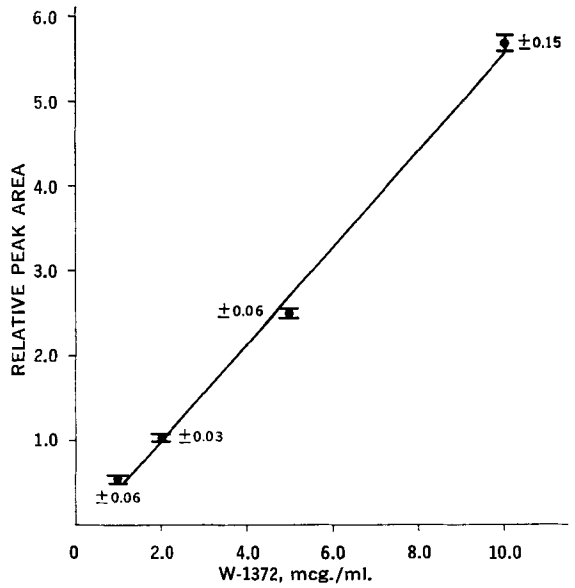

Figure 2—*Relationship between relative peak area and W-1372 concentration in plasma.*

Table II—Blood Concentrations of W-1372 in Several Species after Intravenous Administration[a]

Time, min.	W-1372, mcg./ml.
Dog, 20 mg./kg.	
1	11.9
5	6.1
15	1.1
30	Trace
60	Trace
Squirrel Monkey, 25 mg./kg.	
1	28.9
10	3.9
30	2.0
Cebus Monkey, 25 mg./kg.	
1	9.2
10	3.8
30	Trace

[a] Values are the average of two animals. The amount of drug given is as indicated.

was determined by the relative peak area method, using dibutyl phthalate as the internal standard.

RESULTS AND DISCUSSION

W-1372 can be quantitated gas chromatographically when the relative peak area is used as an index of concentration. The relationship between relative peak area and drug concentration in the range of 1–10 mcg./ml. of plasma is illustrated in Fig. 2. The reproducibility of the procedure, as indicated by the standard error of quintuplicate determinations, is also shown in Fig. 2. The recovery of W-1372 was 98–99%, as shown in Table I.

The extraction procedure effectively separates W-1372 from normal interfering plasma constituents, since determinations in normal plasma of humans (Fig. 1), dogs, or monkeys give little or no blank (<0.1 mcg./ml.). The known major metabolites of W-1372—benzoic acid, hippuric acid, and *N*-γ-phenylpropyl-*N*-benzyloxyamine (3)—do not interfere.

The blood-depletion pattern of W-1372 following intravenous administration was studied in the dog, squirrel monkey, and Cebus monkey. The results (Table II) indicate that the drug is rapidly removed from the blood, with only trace amounts present after 30 min. in the dog and Cebus monkey and a low level present at this time in the squirrel monkey. A previous study (3) has shown that the plasma half-life of radioactivity following oral administration of W-1372-benzyl-^{14}C is 4 hr. in the squirrel monkey and 12 hr. in the dog.

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Mechanism of Action of Retinyl Compounds on Wound Healing I: Structural Relationship of Retinyl Compounds and Wound Healing

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Abstract □ Retinol, retinyl acetate, and retinoic acid promote wound healing. Retinoic acid is most active. Beta-carotene is active, while lycopene is inactive; β-ionone is active and α-ionone is inactive. For full activity, the compound should contain a β-ionone ring, a conjugated double-bond side chain, and a terminal carboxylic acid side chain.

Keyphrases □ Retinyl compounds, activity—structural relationship □ Wound healing—retinyl compound effect □ GLC—separation □ NMR spectroscopy—identity

In a previous report, it was shown that local application of retinol, retinyl acetate, or retinoic acid, dissolved in a nonionic base (NIB), promotes skin wound healing (1). Retinoic acid is relatively more effective than retinol or retinyl acetate. The fact that retinol, retinyl acetate, and retinoic acid are all effective when applied locally on the skin wound indicates that the primary alcohol group is not essential in promoting wound healing. It is of interest, therefore, to study the structural relationship of the other part of the retinol molecule for wound-healing activity. The compounds evaluated in this investigation are β-carotene, lycopene, β-ionone, and α-ionone.

Beta-carotene has the same ring structure and conjugated double-bond hydrocarbon side chain as retinol. It should show activity on wound healing as retinol. Lycopene has essentially the same structure as β-carotene but differs from the latter in not having the closed ring structure at either end of the molecule. Beta-ionone, on the other hand, has the same cyclohexene ring but does not have the same length of hydrocarbon side chain as retinol or β-carotene. Alpha-ionone differs from β-ionone by the position of the double bond in the cyclohexene ring and does not conjugate with the side chain. The structure–activity relationships of these compounds to wound healing are discussed.