SUMMARY

1. A quantitative paper chromatographic assay has been developed for the assay of 4-dehydro-3-keto steroids using an acidified methanolic solution of isonicotinic acid hydrazide.

2. The procedure is used routinely to measure the stability of a number of sex hormones in oil preparations. The assay of 17α-hydroxyprogesterone caproate and testosterone enanthate are described in detail.

3. The method is simple and accurate and permits the simultaneous visual identification and, if necessary, quantitative measurement of any hydrolytic product.

4. No extraction of the sex hormone is required. A simple dilution prior to chromatography is the only sample preparation required.

5. Accuracies in excess of 95% are obtained by simultaneously chromatographing replicates of standard and sample solutions on the same chromatogram.

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Preparation and Pharmacological Properties of Some Benzyl Derivatives of Diphenylacetic Acid

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Benzyl derivatives of diphenylacetic acid were prepared from the starting material 2-chlorodiphenylacetyl chloride. The compounds synthesized were the N-benzyl derivatives of 2-chloro-, 2-hydroxy-, 2-acetoxy-, 2-dimethylaminoethoxy-, and 2-benzylaminodiphenylacetamide; N-benzyl-N-methyl derivatives of 2-chloro- and 2-hydroxy-diphenylacetamide; O-benzyl derivatives of 2-chloro- and 2-hydroxy-diphenylacetamide; and the compound 2-benzylaminodiphenylacetamide. The compounds have limited aqueous solubility so the full extent of the possible pharmacologic actions could not be studied. These agents orally in doses of 1 Gm./Kg. produced no toxic symptoms but the N-benzyl-N-methyl-2-chloro- and the N-benzyl-2-hydroxy-diphenylacetamides inhibited the passage of a charcoal meal, indicating antispasmodic activity similar in potency to that of atropine. Intravenously, the compounds were without cardiovascular or respiratory effects. Intraperitoneally, N-benzyl-2-hydroxy and N-benzyl-2-acetoxy diphenylacetamides showed antipentamethylene tetrazol activity, and one of these also abolished the convulsive actions of electrical shock.

VARIOUS diphenylacetic acid derivatives have long been of interest as antispasmodic agents. The relatively recent literature indicates that certain compounds containing the diphenylacetyl moiety have also been found to possess sedative or anticonvulsant properties. For example, Billman, *et al.* (1, 2), have studied a series of substituted derivatives of 2-aminodiphenylacetamide possessing both anticonvulsant and antispasmodic activity. Among references in the patent literature, N-acetoxy (diphenylacetyl)acetamide (3) is claimed to be an antiepileptic without untoward effects and having extremely low toxicity in animals.

Also apparent from the recent literature is the frequency with which the benzyl group is found, usually as an amide or ester, in compounds with notable properties as sedatives or anticonvulsants. Kushner, *et al.* (4), also observed that many compounds containing a benzylamide moiety possessed pronounced anticonvulsant activity and their study of a variety of such substances resulted in the development of N-benzyl-β-chloropropionamide. N-Benzylmandelamide has

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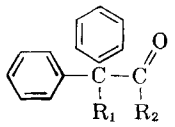
TABLE I.—BENZYL DERIVATIVES OF DIPHENYLACETIC ACID



Compd. No.	R_1	R_2	M. P.,[a] °C.	Recrystn. Solvent	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
I	—Cl	—NH-Benzyl	109–110	Cyclohexane	75.01	5.44	4.21	75.05	5.49	4.10
II	—Cl	—N(CH_3)(Benzyl)	89–90	Cyclohexane	75.52	5.76	4.04	75.55	5.71	4.01
III	—Cl	—O—Benzyl	77–78	Ligroin	74.88	5.09	...	74.90	5.19	...
IV[b]	—OH	—NH—Benzyl	99–100	Cyclohexane	79.46	6.03	4.41	79.45	6.19	4.47
V	—OH	—N(CH_3)(Benzyl)	110.5–111.5	Aq. methanol	79.73	6.39	4.23	79.82	6.34	4.24
VI[c]	—OH	—O—Benzyl	74.5–75.0	Petr. ether	79.22	5.70	...	79.40	5.68	...
VII	—$OCOCH_3$	—NH—Benzyl	169.5–171.5	Isopropanol	76.86	5.89	3.89	76.85	5.97	3.90
VIII	—$OC_2H_4N(CH_3)_2$	—NH—Benzyl	57–58	Isopropanol	77.28	7.26	7.23	77.15	7.33	7.23
IX	—NH—Benzyl	—NH_2	163.0–164.5	Isopropanol	79.72	6.36	8.85	79.82	6.48	8.80
X	—NH—Benzyl	—NH—Benzyl	132.5–133.5	Isopropanol	82.72	6.45	6.89	82.65	6.45	7.05

[a] Melting points are not corrected. [b] Previously described by Lespagnol and Ponthieu (6) who report a melting point of 86°. [c] Previously described by Klinger and Standke (7), m.p. 75–76°.

been reported (5) to be an effective anticonvulsant against electroshock or pentylenetetrazol shock in rats.

These observations suggested the possibility that benzyl amides or esters of substituted diphenylacetic acids might well be active sedative or anticonvulsant agents and, further, might still retain some of the antispasmodic properties more commonly associated with derivatives of these acids.

The compounds of this series, together with pertinent chemical data, are listed in Table I. They were all prepared from 2-chlorodiphenylacetyl chloride which was synthesized from benzilic acid (1). Treatment of this acid chloride with ammonia, benzylamine, or N-benzylmethylamine gave 2-chlorodiphenylacetamide and the substituted 2-chlorodiphenylacetamides (I and II), while reaction with benzyl alcohol produced the benzyl ester (III). The 2-chloro derivatives (I, II, and III), were smoothly converted to the benzilamides (IV and V) or the benzilic acid ester (VI) by warming with aqueous acetone. N-Benzyl-2-chlorodiphenylacetamide (I) reacted with fused sodium acetate in glacial acetic acid to give N-benzyl-2-acetoxydiphenylacetamide (VII), while reaction with sodium 2-dimethylaminoethanolate gave N-benzyl-2-dimethylaminoethoxydiphenylacetamide (VIII). Treatment of 2-chlorodiphenylacetamide and the 2-chloroamide (I) with benzylamine gave the corresponding 2-benzylamino amides (IX and X).

EXPERIMENTAL

2-Chlorodiphenylacetyl Chloride.—This material was prepared by the improved procedure of Billman and Hidy (1) in yields of 70–80%, m.p. 49–50°.

N-Benzyl-2-chlorodiphenylacetamide (I).—Following the procedure of Billman, Ward, and Hidy (2), reaction of 2-chlorodiphenylacetyl chloride with benzylamine in anhydrous ether solution provided an 87% yield of pure N-benzyl-2-chlorodiphenylacetamide.

N-Benzyl-N-methyl-2-chlorodiphenylacetamide (II).—Substitution of N-benzyl-methylamine and dry benzene for benzylamine and anhydrous ether in the above procedure gave a 42% yield of the pure N-benzyl-N-methylamide.

Benzyl 2-chlorodiphenylacetate (III).—Addition of an ether solution of 2-chlorodiphenylacetyl chloride to a chilled ether solution of an equimolar quantity of benzyl alcohol, followed by stirring at room temperature for 24 hours, gave a 45% yield of the benzyl ester after concentration of the reaction solution and crystallization of the oily residue from ligroin.

Benzilic Acid Amides and Ester.—The 2-chlorodiphenylacetamides, (I and II), and the benzyl ester (III), were transformed into the corresponding benzilic acid amides and ester (IV, V, and VI) in 84, 75, and 68% yields, respectively, by refluxing the chloro compounds for 8–12 hours in a 1:3 acetone-water mixture. The products were isolated by concentration of the reaction mixtures and recrystallization of the solid residues from appropriate solvents.

N-Benzyl-2-acetoxydiphenylacetamide (VII).—This compound was prepared by reacting N-benzyl-2-chlorodiphenylacetamide with fused sodium acetate in glacial acetic acid according to the procedure of Emerson, *et al.* (8). The product was isolated in 48% yield by pouring the reaction mixture over ice and recrystallizing the precipitated solid several times from isopropanol.

N-Benzyl-2-dimethylaminoethoxydiphenylacetamide (VIII).—Dropwise addition of a solution of N-benzyl-2-chlorodiphenylacetamide in dry benzene to a benzene suspension of an equimolar quantity of sodium dimethylaminoethanolate, prepared from sodium and dimethylaminoethanol, was followed by refluxing of the reaction mixture for 12 hours. After filtering off the precipitated sodium chloride, the benzene solution was extracted with dilute

hydrochloric acid and the crude product was precipitated by addition of sodium hydroxide solution to the combined acidic extracts. Several recrystallizations from isopropanol afforded a 39% yield of the pure basic ether. The hydrochloride salt of the product decomposes at 192° after recrystallization from a mixture of methanol, isopropanol, and ether. It is not appreciably soluble in water.

2-Chlorodiphenylacetamide.—Addition of gaseous ammonia to a chilled ether solution of 2-chlorodiphenylacetyl chloride, following the method of Bickel (9), gave a pure product, melting at 118–119°, in 90% yield after recrystallization from toluene.

2-Benzylaminodiphenylacetamide (IX).—Benzylamine was reacted with 2-chlorodiphenylacetamide in benzene solution according to the procedure of Billman, Ward, and Hidy (2) to afford a 38% yield of pure product after several recrystallizations from isopropanol.

N-Benzyl-2-benzylaminodiphenylacetamide (X).—This compound was prepared by the above procedure from N-benzyl-2-chlorodiphenylacetamide and benzylamine in 45% yield.

PHARMACOLOGY

Pharmacologic studies were carried out on these compounds using dogs, cats, hamsters, and rats. The full extent of the possible actions of these chemicals could not be determined due to their restricted solubility in aqueous solutions. None of the agents were soluble to the extent of 1% in water or in 25% aqueous dimethylacetamide, and only compounds I and IV appeared to be soluble to the extent of 1% in ethylene glycol at 35°. Wherever possible, the compounds were suspended in water with gum acacia. Toxicity in rats could not be determined.

Doses of 1 Gm./Kg. given orally to rats produced no toxic symptoms. The influence of these agents on the intestinal motility of the small intestine was measured as the inhibition of passage of a charcoal meal, using the method of Macht and Gose. Each compound was suspended in an aqueous solution of 10% charcoal and 10% gum acacia. One milliliter of this suspension was given by stomach tube, and 1 hour later the rats were sacrificed, the intestines removed, and the distance traversed by the charcoal and the length of the intestines were measured. The ten controls averaged 69% with a standard deviation of ±6%. Atropine sulfate in doses of 0.1, 1.0, 2.0, and 3.0 mg./Kg. in 5 animals each was used as a standard of reference, giving the means and standard deviations as follows: 67±5, 62±5, 52±4, 50±4%, respectively. A dose of atropine *vs.* the percentage of the total distance the charcoal meal traversed curve was plotted. The effectiveness of the compounds tested was given in terms of equivalent effective doses of atropine. The test compounds were given in doses up to 0.75 Gm./Kg., and compounds II and VI influenced the intestinal motility. At this dose level, the activity of II and VI were equivalent to that of 1.0 and 0.2 mg./Kg. of atropine, respectively. At a dose level of 1.0 Gm./Kg. with these two agents, the activity of II was equivalent to 3.0 mg./Kg. of atropine and that of VI was equal to 2.0 mg./Kg. of atropine.

Blood pressure studies were made on the pentobarbitalized dog and cat following intravenous injections of 2.0 mg./Kg. of the chemicals in 25% ethanol in isotonic saline. There was only a minimal effect of these compounds on the mean arterial blood pressure; an equivalent amount of 25% ethanol in isotonic saline was used as a control and was found to be without effect on the blood pressure.

These compounds do not appear to be well absorbed orally because of lack of solubility and toxicity, so the intraperitoneal route of injection was used for the anticonvulsant testing. The agents were dissolved in hot 50% ethanol and cooled to 40° just before injection, and the animals in groups of 10 were tested 1 hour later. Control animals given 50% ethanol in saline in the volumes used in the experiments did not show central nervous system effects nor did they differ in convulsant thresholds when compared to saline-treated animals. Anticonvulsant studies were carried out first by the rat subcutaneous pentylenetetrazol test. This demonstrated the activity of the compound being tested to block the convulsions produced by 100 mg./Kg. pentylenetetrazol given subcutaneously. Phenobarbital sodium was used as the standard of comparison.

Due to limited solubility, compounds IV and VII were injected in doses of 100 mg./Kg., while the rest of the compounds were administered in doses of 40 mg./Kg. Only compounds IV and VII showed antipentylenetetrazol activity in doses of 40 mg./Kg. In doses of 100 mg./Kg., compound VII exhibited a minimal activity, while compound IV elicited an effectiveness similar to hypnotic doses of phenobarbital. The second method used was the supramaximal electrical shock test. This showed the ability of the compounds to abolish the hind limb extensor reflex produced by a current of 150 ma. delivered for 0.3 sec. to the brain via the ear electrodes, using the rat. Only compound IV, in doses of 100 mg./Kg., was effective in this respect by abolishing the hind limb extensor reflex in 4 out of the 10 animals.

The "wild" hamster is useful in determining sedative and tranquilizer effects, since the actions of the barbiturates and reserpine are easily differentiated when this animal is hungry and made angry by being picked up by the tail. Intraperitoneal injections of 40 mg./Kg. of these compounds had no effect on this animal.

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