in vacuo left an oily yellow residue. The residue was extracted with 250 ml. of boiling absolute ether, and the extract was treated with Darco G-60, filtered, and chilled to give colorless needles of 5-allyl-3-methylhydantoin. Recrystallization once from absolute ether, and once from ether–acetone gave 11.0 g. (71% yield) m.p. 90–92°.

Anal. Calcd. for $C_7H_{10}N_2O_2$: C, 53.53; H, 6.54; N, 11.87. Found: C, 54.2; H, 6.6; N, 18.1.

5-(3-Chloromercuri-2-methoxy-1-propyl)-3-methylhydantoin.—Solutions of 5-allyl-3-methylhydantoin (3.5 g., 0.023 mole) in 75 ml. of hot methanol, and 4.6 g. (0.023 mole) of mercuric acetate in 75 ml. of hot methanol were mixed, and treated with 2 drops of concd. nitric acid. After 5 min. the clear solution was treated with 5.8 g. (0.1 mole) of sodium chloride in 25 ml. of water. The product, obtained from the cooled solution as white needles, was recrystallized once from aqueous methanol to give 5.2 g. (55% yield), m.p. 142–154°.

Anal. Calcd. for $C_8H_{13}ClHgN_2O_3$: C, 22.81; H, 3.11; N, 6.65; Hg, 47.62. Found: C, 22.6; H, 3.2; N, 6.5; Hg, 46.4.

Acknowledgments.—We wish to thank Dr. Robert L. Clarke for his interest and helpful discussions, and Mr. Leon P. Duprey and Miss Nancy D. Harvey for the toxicity screening.

Potential Uricosuric Agents Derived from 1,3-Diphenylbarbituric Acid

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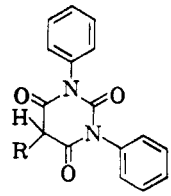
Received June 2, 1961

1,3-Diphenylbarbituric acids with 5-acyl, carbamoyl and alkyl substituents have been prepared and tested for possible uricosuric activity as indicated by their ability to cause retention of phenol red in the circulation of the rat. The compounds were 67 to 128% as active as phenylbutazone. The synthesis of 1,3-diphenylbarbituric acid has been investigated and a by-product, 5-(α-carbethoxyacetyl)-1,3-diphenylbarbituric acid, characterized.

Compounds containing an easily replaceable hydrogen as part of a β-dicarbonyl system have divergent biological actions. These actions include antibacterial (*e.g.*, tetracyclines), anthelmintic (*e.g.*,

filixic acid from *Aspidium*), anticoagulant (*e.g.*, 4-hydroxycoumarins and indane-1,3-diones), anti-inflammatory (*e.g.*, phenylbutazone) and uricosuric (sulfinpyrazone).[1]

In the present work, 1,3-diphenylbarbituric acid has been employed as the parent β-dicarbonyl system. The derivatives investigated have been 5-acyl, carbamoyl, and alkyl 1,3-diphenylbarbituric acids (I).



I, R = acyl, carbamoyl, alkyl

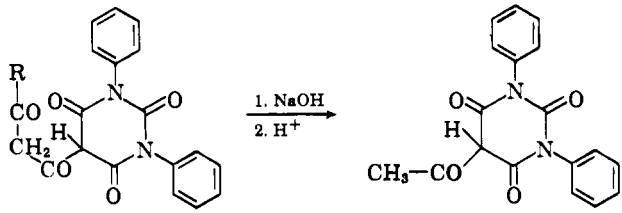
It was of interest to know whether these compounds might have either more uricosuric activity or a more unusual pharmacologic action than certain 1,2-diphenyl-3,5-pyrazolidinediones from which they might be considered derived by the insertion of a carbonyl function between the two nitrogen atoms.

1,3-Diphenylbarbituric acid has been prepared by the reaction of a carbanilide-chloroform mixture with either malonyl chloride or with malonic acid and phosphorus oxychloride.[2] 5-Acetyl-1,3-diphenylbarbituric acid, m.p. 150°, was mentioned[2] as a by product—which was not analyzed—in the preparation with malonic acid and phosphorus oxychloride. The acetyl derivative was separated by crystallization from ethanol after a work-up which included an ethanol quench to decompose excess phosphorus oxychloride and a sodium carbonate extraction to separate unreacted carbanilide.[2] In our hands, the malonic acid–phosphorus oxychloride procedure gave 40% unchanged carbanilide and a sodium carbonate soluble portion which melted at 148–154°. The melting point was unchanged upon fractional crystallization from ethanol. When the product was dissolved in warm benzene, and the solution cooled, 1,3-diphenylbarbituric acid, m.p. 238-242°, separated (13%). Upon concentration and dilution of the benzene liquor with heptane there was obtained a second

(1) Detailed accounts of drugs of these types may be found in A. Burger, "Medicinal Chemistry," 2nd ed., Interscience Publishers, Inc., New York, N. Y., 1960.

(2) M. A. Whitely, *J. Chem. Soc.*, **91**, 1330 (1907).

crop, m.p. 148–154°. This was recrystallized from isopropyl acetate to furnish a 30% yield of material with analysis for II-b and melting at 160–163°. Confirmation of this structure was obtained by saponification, then decarboxylation to 5-acetyl-1,3-diphenylbarbituric acid, m.p. 149°, (III). The latter also was prepared by acetylation of 1,3-diphenylbarbituric acid.



IIa, R = Cl
b, R = OC_2H_5

III

Compound II-b may have been formed during the phosphorus oxychloride–ethanol quench from a precursor such as II-a, formed by acylation of 1,3-diphenylbarbituric acid with malonyl chloride. Failure of the earlier workers to isolate II-b may have been the result of hydrolysis of an intermediate such as II-a to a β-keto acid with subsequent decarboxylation to form 5-acetyl-1,3-diphenylbarbituric acid. It should be noted that the chloroform used in our experiments was rendered alcohol-free by drying over calcium chloride for 24 hours, while the earlier workers employed sodium-dried chloroform.[2]

As an intermediate, 1,3-diphenylbarbituric acid was prepared by the reaction of carbanilide with malonyl chloride in chloroform in 69% yield. None of the 5-acyl derivatives was isolated when this method was employed. It may be that a more facile reaction ensues, and thus the large excesses of malonyl chloride relative to formed 1,3-diphenylbarbituric acid are avoided.

The 5-acyl derivatives (III, IV) of Table I were prepared readily by the reaction of an acid chloride with sodium 1,3-diphenylbarbiturate in tetrahydrofuran. That C-acyl compounds were isolated is shown by the solubility of the products in 0.1 *N* sodium hydroxide. In addition, crude products gave colored precipitates with methanolic cupric acetate. In practice sodium 1,3-diphenylbarbiturate was prepared by the addition of 1,3-diphenylbarbituric acid to an aqueous solution of an equivalent amount of sodium hydroxide. Con-

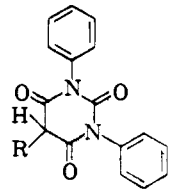
centration yielded the tetrahydrofuran soluble sodium salt.

By a method previously reported,[3] 5-carbamoyl-1,3-diphenylbarbituric acid (V) was prepared by the reaction of 1,3-diphenylbarbituric acid with urea.

Reductive alkylation of 1,3-diphenylbarbituric acid by a general procedure reported by Alexander and Cope[4] furnished the 5-alkyl derivatives (VI–VIII) of Table I. It was not possible to isolate a pure 5-butylidene derivative by the reaction of 1,3-diphenylbarbituric acid with butyraldehyde although a variety of reaction conditions was investigated. 1,3-Diphenyl-2-thiobarbituric acid has been treated with heptanal to form colored products which were not iso-

TABLE I

5-SUBSTITUTED-1,3-DIPHENYLBARBITURIC ACIDS



Cpd.		Yield, % anal. pure	M.p., °C.	λ_{max} 0.1 *N* NaOH–EtOH	
III	CH_3CO—	68	144–146	270, 247	16,380, 13,800
IV	$CH_3(CH_2)_2CO$—	76	123.5–124.5	273, 249	16,000, 15,270
V	H_2NCO—	53	230–232	253	21,700
VI	$CH_3(CH_2)_2$—	90	147–149	271	21,500
VII	$CH_3(CH_2)_3$—	98	118–123	276	19,200
VIII	$CH_3(CH_3)_6$—	86	130–131	271	23,200

	Analyses, %					
	Calcd.			Found		
Cpd.	C	H	N	C	H	N
III	67.07	4.38	8.69	66.86	4.50	8.65
IV	68.56	5.18	8.00	68.74	5.27	8.03
V	63.15	4.05	13.00	63.31	4.24	12.96
VI	70.79	5.63	8.69	70.82	5.67	8.87
VII	71.41	5.99	8.33	71.24	5.86	8.05
VIII	72.99	6.93	7.40	72.86	6.84	7.25

(3) H. C. Scarborough, *J. Org. Chem.*, **26**, 2579 (1961).

(4) E. R. Alexander and A. C. Cope, *J. Am. Chem. Soc.*, **66**, 886 (1944).

lated[5] and has been condensed with both aromatic aldehydes and ketones.[6]

Chemical Experimental[7]

Reaction of Malonic Acid and Carbanilide with Phosphorus Oxychloride. A. 1,3-Diphenylbarbituric Acid (I).—A mixture of 30 g. (0.142 mole) of carbanilide, 15 g. (0.144 mole) of recrystallized malonic acid, and 45 g. of phosphorus oxychloride in 150 ml. of calcium chloride-dried chloroform was refluxed on the steam-bath for 7 hr. and then concentrated under reduced pressure to a red syrup. After partial cooling the syrup was suspended in 150 ml. of absolute ethanol. After standing overnight the solution was cautiously diluted with water to 300 ml. and chilled to furnish an orange solid which was collected. The solid was stirred with 1.4 l. of 5% aqueous sodium carbonate for 1 hr. and the insoluble solid separated (carbanilide, 12 g., 40% recovery). The liquor was acidified and the precipitated solid collected and dried to furnish 25.3 g., m.p. 141–155°. The latter material was dissolved in 500 ml. of benzene and the solution treated with charcoal. After the addition of 25 ml. of heptane to prevent freezing the solution was cooled in an ice bath. The crystallized solid was collected and recrystallized from benzene to furnish 5.1 g. (13%), m.p. 238–242°, lit.[2], m.p. 238°, $\lambda_{max}^{0.1\ N\ NaOH}$ 264 (ϵ = 26,400)).

Anal. Calcd. for $C_{16}H_{12}N_2O_3$: C, 68.56; H, 4.32; N, 10.00. Found: C, 68.58; H, 4.37; N, 9.92.

B. 5-(α-Carbethoxyacetyl)-1,3-diphenylbarbituric Acid (II-b).—Concentration of the benzene liquors from the above experiment gave an oil which was crystallized from ethanol and recrystallized from isopropyl acetate to furnish 9 g. (30%), m.p. 160–163°, $\lambda_{max}^{0.1\ N\ NaOH}$ 277 and 247 (ϵ = 13,200 and 10,620).

Anal. Calcd. for $C_{21}H_{18}N_2O_6$: C, 63.95; H, 4.60; N, 7.10. Found: C, 63.90; H, 4.83; N, 7.14.

5-Acetyl-1,3-diphenylbarbituric Acid (III). A. Saponification of 5-(α-Carbethoxyacetyl)-1,3-diphenylbarbituric Acid.—A solution of 3.1 g. (0.01 mole) of II-b in 30 ml. of 4% aqueous sodium hydroxide was boiled for 3 min. A small amount of precipitated material was separated and the liquor acidified to precipitate a buff-colored solid. The solid (1.6 g.) was dried and recrystallized by the addition of heptane to a butanone solution and by the addition of isopropyl ether to a solution in isopropyl acetate, m.p. 149° (dec., slow rate of heating).

B. Acetylation of 1,3-Diphenylbarbituric Acid.—To 20 ml. of 0.5 *N* sodium hydroxide (0.01 mole) was added 2.8 g. (0.01 mole) of 1,3-diphenylbarbituric acid. The mixture was warmed to furnish a solution which was concentrated under reduced pressure to yield a crystalline residue. After drying by the addition and removal of two portions of absolute ethanol the salt was dissolved in 25 ml. of tetrahydrofuran. To this solution was added 0.85 ml. (0.012 mole) of acetyl chloride.

(5) K. Taüfel and R. Zimmermann, *Naturwissen.*, **47**, 133 (1960).

(6) M. A. Whitely, *Proc. Chem. Soc.*, **25**, 121 (1909).

(7) All melting points are uncorrected. Microanalyses were performed by Mr. Clarence Kennedy of the Mead Johnson Research Center.

The solution became warm and a solid precipitated. After stirring for 3 hr. the mixture was concentrated and the residual gum dissolved in 10% sodium carbonate solution. Acidification precipitated a white solid which was purified as above to furnish 2.2 g. (68%), m.p. 149°, with no depression on admixture with material prepared by saponification.

5-Butyryl-1,3-diphenylbarbituric Acid (IV).—This material was prepared as above, except that a solution of butyryl chloride in tetrahydrofuran was added with stirring and cooling to a solution of the salt in tetrahydrofuran. The product was purified by recrystallization from aqueous methanol and from a solution of 2 parts heptane and 1 part cyclohexane.

5-Carbamoyl-1,3-diphenylbarbituric Acid (V).—A solution of 10.7 g. (0.0383 mole) of 1,3-diphenylbarbituric acid and 4.6 g. (0.0766 mole) of urea in 12 ml. of 1-methyl-2-pyrrolidone was heated at 150–155° for 17 minutes. The reaction solution was diluted while warm to 300 ml. with water. The mixture was adjusted to pH 3 with hydrochloric acid. After cooling, the solid product was collected and then recrystallized from aqueous methanol and from benzene to furnish 6.7 g. (53%), m.p. 230–232° with good depression on admixture with 1,3-diphenylbarbituric acid.

5-Alkyl-1,3-diphenylbarbituric Acids (VI–VIII).—A solution of 5.6 g. (0.02 mole) of 1,3-diphenylbarbituric acid, 0.022 mole of freshly distilled aldehyde and 4 drops of piperidine in 100 ml. of glacial acetic acid was shaken under hydrogen with 10% palladium-on-carbon. Hydrogen consumption ceased at the theoretical value in about 2 hr. The catalyst was separated and the solution concentrated under reduced pressure. The product was crystallized by the addition of 0.1 *N* hydrochloric acid to a solution of the residue in methanol. Recrystallizations from methanol and from benzene or cyclohexane furnished pure materials.

Pharmacologic Studies

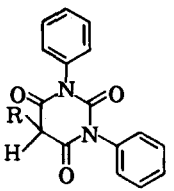
The effects of compounds in Table II on the retention of phenol red in the rat's circulation were determined by a modification of a method which has been described by Kreppel.[8] Groups of 7–10 adult rats (180–220 g.) received the test compound, 100 mg./kg. subcutaneously, 15 minutes prior to their being anesthetized with diallylbarbituric acid–urethan (0.5 ml./kg., i.p.). Fifteen minutes after injection of the anesthetic each rat received phenol red via the caudal vein (75 mg./kg., as a 1.5% solution in 0.9% NaCl). Blood samples were taken from the retro-orbital plexus[9] into heparinized capillary tubes at 10, 30, and 60 minutes after injection of the dye. After centrifugation a 0.02 ml. aliquot of plasma was transferred by a Sahli pipet to a Coleman cuvette (12 × 75 mm.) containing 1.5 ml. of 0.9% NaCl. Color was developed by addition of 0.05 ml. of 0.1 *N* NaOH, and read at 540 mμ in a Coleman Junior Spectrophotometer against a blank containing plasma, saline, and 0.05 ml. of 0.1 *N* HCl. This procedure provided for the detection of 20–120 μg. of phenol red/cuvette. Results were calculated as mg. of phenol red remaining/ml. plasma/time interval and are reported in Table II both as the absolute amounts of phenol red and as

(8) E. Kreppel, *Med. exp.*, **1**, 285 (1959).

(9) B. N. Halpern and A. Picaud, *Compt. Rend. Soc. Biol. Paris*, **145**, 1465 (1951).

TABLE II

EFFECTS OF CERTAIN 1,3-DIPHENYLBARBITURIC ACID DERIVATIVES ON RETENTION OF PHENOL RED IN THE RAT



Compound	R	pK_a[a]	Phenol red remaining[b] 10	30	60	Percentage of phenylbutazone activity 10	30	60
None (untreated control)	.....	..	2.03[c] ± 0.08	0.82 ± 0.06	0.26 ± 0.04	...	...	...
Phenylbutazone		5.05	2.11 ± 0.06	1.29 ± 0.09	0.57 ± 0.06	100	100	100
III	CH_3CO—	4.23	2.10 ± 0.05	1.28 ± 0.08	0.59 ± 0.06	99	99	104
IV	$CH_3(CH_2)_2CO$—	4.50	2.21 ± 0.14	1.20 ± 0.08	0.52 ± 0.08	105	93	91
V	H_2NCO—	5.65	2.37 ± 0.09	1.25 ± 0.08	0.38 ± 0.05	113	97	67
VI	$CH_3(CH_2)_2$—	3.80	2.19 ± 0.09	1.20 ± 0.08	0.56 ± 0.06	104	93	98
VII	$CH_3(CH_2)_3$—	4.15	2.21 ± 0.17	1.37 ± 0.08	0.73 ± 0.11	105	106	128
VIII	$CH_3(CH_2)_6$—	[d]	2.23 ± 0.10	1.29 ± 0.12	0.55 ± 0.06	106	100	96

[a] Determined by titration in 50% aqueous methanol. K. Wallenfels and H. Sund, in *Arzneimittel-Forsch.*, 9, 81 (1959), report a pK_a of 4.89 for phenylbutazone by titration in 50% alcohol. As determined by the ultraviolet absorption of a dilute aqueous solution, the pK_a of phenylbutazone is 4.50 (ref. 10). [b] Mg. of phenol red remaining per ml. of plasma at indicated time intervals (min.); 7–10 rats per group; dose was 100 mg./kg. s.c. [c] Mean ± S.E. [d] Insoluble in aqueous methanol.

the percentage of phenylbutazone activity.

These six compounds have 67 to 128% of the phenol red retentive activity of phenylbutazone (Table II). In a series of phenylbutazone analogs it is reported[10] that the more acidic compounds possess in turn a greater degree of uricosuric activity. Since compound VII, which is structurally the most similar of this series to phenylbutazone, is over-all the most active of the group, it appears that structural characteristics as well as acidity may be critical determinants of this ability to induce in the rat's circulation retention of phenol red. Furthermore, the most acidic compound in this series (VI) is no more active than phenylbutazone. However, the least acidic compound (V) is significantly less active than phenylbutazone (P = 0.02–0.05) at the 60 minute determination. That more than acidity is important in endowing a compound with clinical uricosuric activity is evidenced by the efficacy of zoxazolamine.[11]

The data reported herein do not indicate that any of these six compounds would be more effective uricosuric agents in man than those currently available. Measurement of phenol red retention in the rat is not a direct index of therapeutic uricosuric activity in man, but that such animal data as these might be expected to be predictive of clinical utility is seen in reports by Brodie *et al.*[12] and Burns *et al.*,[13] which describe the inhibition of excretion of phenol red by phenylbutazone and certain analogs in man.

The data in Table III indicate that none of these compounds exhibited typical barbiturate action in the rat in that high dosages were required for activity. Under similar conditions sodium pheno-

TABLE III

Compound	Dose.[a] mg./kg. s.c.	Vehicle	Remarks
III	300	Saline[b]	No evidence of neurologic deficit
IV	400	0.5% Methocel[c]	Depression within 75 min.: tremors; 3 deaths
V	300	Same	No evidence of neurologic deficit
VI	300	Saline	No evidence of neurologic deficit
VII	300	Saline	Mild depression within 75 min.
VIII	400	0.5% Methocel	No evidence of neurologic deficit

[a] Administered to 10 rats (140–160 g.). [b] Dissolved in 1 N NaOH; pH adjusted to 7.5–7.8 with 1 N HCl; vol. adjusted with 0.9% NaCl. [c] Suspended in 1% Methocel and diluted 1:1 with 0.9% NaCl.

(10) J. Burns, T. F. Yu, L. Berger, A. Ritterband, A. B. Gutman, and A. B. Brodie, *J. Pharmacol. Exp. Therap.*, **119**, 136 (1957).

(11) G. D. Kersley and A. R. Gibbs, *Ann. rheum. Dis.*, **19**, 351 (1960).

(12) B. B. Brodie, T. F. Yu, J. J. Burns, T. Chenkin, B. C. Paton, J. M. Steele, and A. B. Gutman, *Proc. Soc. exp. Biol., N.Y.*, **86**, 884 (1954).

(13) J. J. Burns, T. F. Yu, A. Ritterband, L. M. Perel, A. B. Gutman, and B. B. Brodie, *J. Pharm. Exp. Therap.*, **119**, 418 (1957).

barbital produced profound depression of the central nervous system at a dose of 100 mg./kg.

Acknowledgment.—The authors wish to thank Mr. Jerry L. Compton for synthesis of some of the intermediate compounds, and Messrs. Norbert E. Adler and John P. Braselton for the pharmacologic studies.

Large Scale Preparation and Purification of the Vasopressor Polypeptide, Substance A[1]

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Revised Manuscript Received June 2, 1961

A procedure for the large scale preparation of substance A is described. By means of precipitation techniques and chromatography on carboxymethylcellulose we have been able to purify substance A 70-fold. Using paper electrophoresis substance A has been shown to move as a discrete substance and by means of paper electrophoresis it has been shown to be an amphoteric substance with an isoelectric pH between 6–7.

We have reported previously that a pharmacologically active polypeptide provisionally designated as substance A can be produced by incubating an α-amylase preparation with fraction IV-4 of human plasma protein.[3,4] Chemical and pharmacological comparison with other known polypeptides led to the conclusion that substance A was very similar to angiotensin.[4] However, efforts to characterize adequately this material have been handicapped by inadequate amounts of substance A and by lack of material of sufficient purity

(1) Supported by a grant from the National Heart Institute, U. S. Public Health Service (Grant H-4873). A preliminary report of this work was presented at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, University of Washington, Seattle, Aug. 22–25, 1960.

(2) This investigation was supported by a Senior Research Fellowship (SF-72) from the Public Health Service.

(3) C. G. Huggins and E. J. Walaszek, *Proc. Soc. Exp. Biol. N. Y.*, **100**, 100 (1959).

(4) E. J. Walaszek and C. G. Huggins, *J. Pharmacol.*, **126**, 258 (1959).