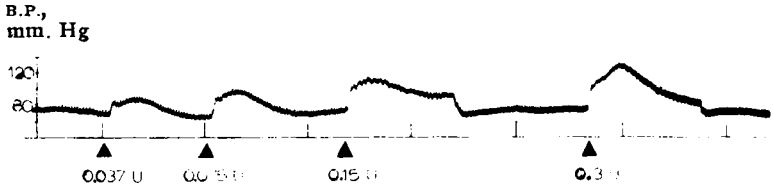

Fig. 3.—Effects of increasing doses of vasopressin after arrest of tachyphylaxis in the reserpinized dog. All doses were in units per kilogram. Time marker: 10-minute intervals.

flexes. Once tachyphylaxis was arrested, dose-response effects were observable. This observation suggests that the use of reserpinized dogs for the bioassay of vasopressin should be investigated.

Our results with angiotensin agreed with those of Page, *et al.* (14). In the pure synthetic form, it was not tachyphylactic and did not affect vasopressin tachyphylaxis. Although both angiotensin and vasopressin are octapeptides, the differences between their structural formulas are sufficient to make it not surprising that there was no cross tachyphylaxis. For the same reason it was expected that, though renin blocked the pressor response to angiotensin, it would not affect the pressor response to vasopressin.

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Synthesis of Potential Antineoplastic Agents I

By WILLIAM D. ROLL

Four new derivatives of *o,o'*-diphenamide have been prepared to evaluate their anticarcinogenic activity: *o,o'*-bis(*N*-2-mesyloxyethyl)diphenamide, *o,o'*-bis[*N*-2-(3-chloropropionamido)ethyl]diphenamide, *o,o'*-bis[*N*-2-(3-bromopropionamido)-ethyl]diphenamide, and *o,o'*-bis(*N*-2-mercaptoethyl)diphenamide.

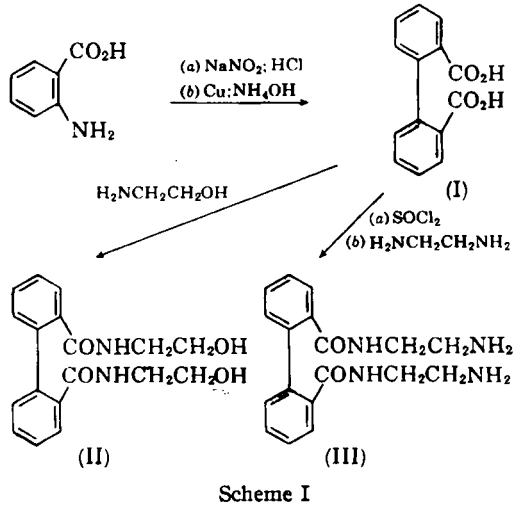
A SERIES OF derivatives of diphenamide was synthesized for the purpose of evaluating their possible antineoplastic activity.

Carbon and co-workers (1) reported that various bis-amides show wide antitumor activity. Baker (2) suggested that the configuration of the "back side" of a molecule may be altered in major ways to give better irreversible bonding and perhaps enhance activity. This report describes the synthesis of some related amides which are structural analogs of diphenamide and which might possibly function as exoalkylating irreversible inhibitors.

The synthetic procedure used for the preparation of these analogs of *o,o'*-diphenamide may be briefly outlined as follows. *o,o'*-Diphenic acid (I), synthesized by the procedure described by Atkinson and Lawler (3), was converted to *o,o'*-bis(*N*-2-hydroxyethyl)diphenamide (II), by a procedure similar to that described by Wenker (4). Treatment of II with methanesulfonyl chloride yielded *o,o'*-bis(*N*-2-mesyloxyethyl)diphenamide (IV).

Treatment of I with thionyl chloride gave *o,o'*-diphenoyl chloride (VII) (5, 6). The acyl halide reacted with ethylene diamine at low temperatures to give the amide *o,o'*-bis(*N*-2-aminoethyl)diphenamide (III). Acylation of the amino analog (III) with 3-chloropropionyl chloride or ethyl 3-chloropropionate and 3-bromopropionyl chloride or methyl 3-bromopropionate gave *o,o'*-bis[*N*-2-(3-chloropropionamido) ethyl]diphenamide and *o,o'*-bis[*N*-2-(3-bromopropionamido)ethyl]diphenamide, compounds V and VI, respectively.

To introduce the mercaptoethyl side chain, diphenic acid (I) was converted to the acyl halide (VII) initially. It was allowed to react with



Scheme I

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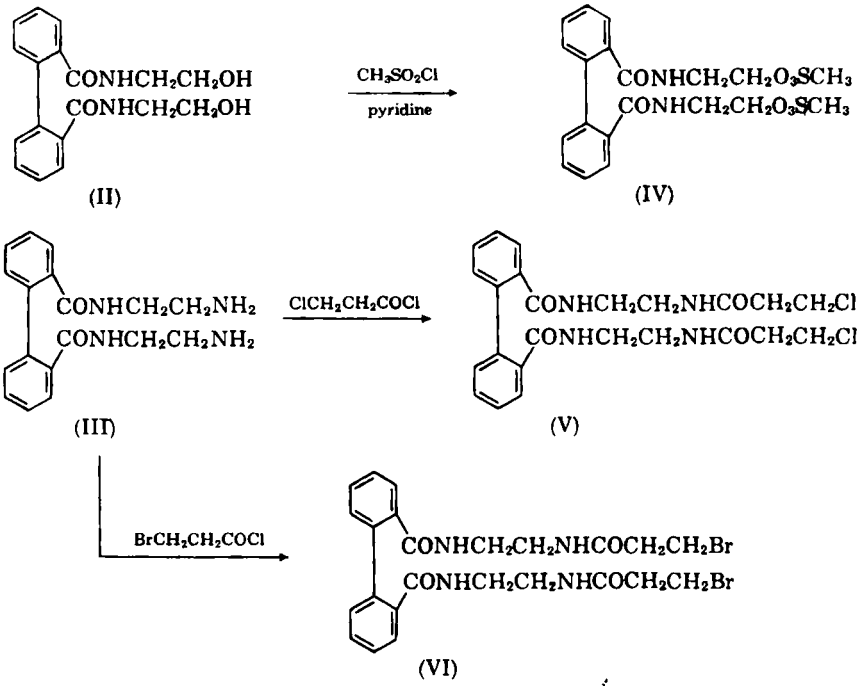


Scheme II

sodium hydrosulfide to give *o,o'*-thiodiphenic acid (VIII) by a procedure similar to that described in the literature for the synthesis of thiobenzoic acid (7–9). The thio acid gave *o,o'*-bis(*N*-2-mercaptoethyl)diphenamide (IX) on treatment with ethylenimine (10) by a procedure analogous to that described by Kuhn and Quadbeck (11).

EXPERIMENTAL

The sequence of synthetic reactions is shown by Schemes I, II, and III.

o,o'-Diphenic Acid (I).—The procedure used for the synthesis of diphenic acid was that described in "Organic Syntheses" (3).

o,o'-Bis(N-2-hydroxyethyl)diphenamide (II).—A mixture of 4.8 Gm. (0.02 mole) of I and 2.4 Gm. (0.04 mole) of 2-aminoethanol was heated in an open flask until the loss in weight corresponded to 2 moles of water. The crude material was crystallized from absolute methanol to give 4.4 Gm. (68%) of product melting at 130–131°. The compound did not form a salt with HCl, and the infrared spectrum showed a broad band at 3.1 μ. This would indicate that the amide was formed rather than the ester.

Anal.—Calcd. for $C_{18}H_{20}N_2O_4$: C, 65.84; H, 6.14; N, 8.53. Found: C, 66.08; H, 6.10; N, 8.48.

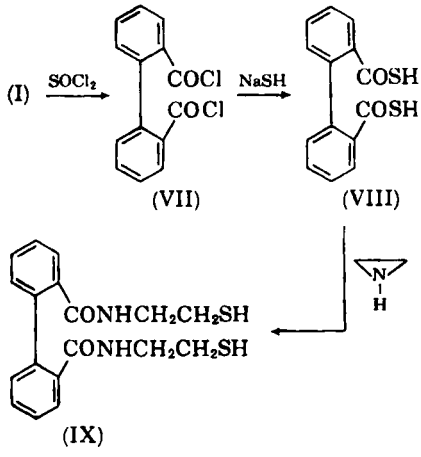
o,o'-Bis(N-2-mesyloxyethyl)diphenamide (IV).—A solution of 1.3 Gm. (0.004 mole) of II in 2.5 ml. of anhydrous pyridine was cooled to 0°. A 1.4-Gm. (0.012 mole) quantity of methanesulfonyl chloride, previously cooled to 0°, was added to this in a dropwise manner. The mixture was allowed to stand for 1 hour, then poured with rapid stirring into ice water. The product was removed on a cold Buchner funnel and washed several times with cold water. The product was crystallized from absolute methanol to give 0.8 Gm. (42%) of product melting at 192–193°.

Anal.—Calcd. for $C_{20}H_{24}N_2O_8S_2$: C, 49.57; H, 4.99; N, 5.78; S, 13.23. Found: C, 49.65; H, 5.10; N, 5.70; S, 13.34.

o,o'-Bis(N-2-aminoethyl)diphenamide (III).—To a cold (0°) 2.4-Gm. (0.04 mole) solution of ethylenediamine and 5% aqueous sodium hydroxide was added a chloroformic solution containing 4.8 Gm. (0.02 mole) of *o,o'*-diphenoyl chloride (VII). The mixture was shaken vigorously for 5 minutes, and the crude amine was removed by filtration. The crude material was crystallized twice from dry chloroform to give 4.0 Gm. (61%) of product melting at 220–221°.

Anal.—Calcd. for $C_{18}H_{22}N_4O_2$: C, 66.23; H, 6.79; N, 17.17. Found: C, 65.98; H, 6.83; N, 17.30.

o,o'-Bis[N-2-(3-chloropropionamido)ethyl]diphenamide (V).—A solution of 2.0 Gm. (0.016 mole) of 3-chloropropionyl chloride in dry chloroform was



Scheme III

added dropwise to a 2.6-Gm. (0.008 mole) solution of the amine (III) in chloroform at room temperature. When the addition of the acyl halide was complete, the reaction mixture was refluxed until the evolution of hydrogen chloride had ceased. The reaction mixture was concentrated *in vacuo* and the crude amide removed by filtration. The crude product was placed in a Soxhlet apparatus and extracted with dry chloroform for several hours. The product melted at 225–226°, yield 3.2 Gm. (79%).

Anal.—Calcd. for $C_{24}H_{28}Cl_2N_4O_4$: C, 56.81; H, 5.56; Cl, 13.98; N, 11.02. Found: C, 57.01; H, 5.48; Cl, 14.01; N, 11.22.

o,o′-Bis[N-2-(3-bromopropionamido)ethyl]diphenamide (VI).—The same procedure described above for the preparation of the chloropropionamide analog was used. To a 2.6-Gm. (0.008 mole) solution of the amine (III) in dry chloroform was added 2.5 Gm. (0.016 mole) of 3-bromopropionyl chloride (in dry chloroform). A slightly longer reflux period was necessary for completion of the reaction than with compound V. The product weighed 3.4 Gm. (57%) and melted at 244–245°.

Anal.—Calcd. for $C_{24}H_{28}Br_2N_4O_4$: C, 48.34; H, 4.73; Br, 26.80; N, 9.40. Found: C, 48.50; H, 4.65; Br, 26.74; N, 9.44.

o,o′-Diphenoyl Chloride (VII).—The procedure used for the synthesis of diphenoyl chloride and the results obtained have been previously described (5, 6).

o,o′-Thiodiphenic Acid (VIII).—A 2.8-Gm. (0.01 mole) quantity of diphenoyl chloride (VII) was added to a solution of sodium sulfhydrate, prepared from 3.7 Gm. (0.04 mole) of NaSH in 25 ml. of distilled water. The mixture was stirred and heated on a steam bath for 3 hours. It was cooled to 0°, surrounded by an ice bath, and slowly acidified with a cold solution of 3 *N* hydrochloric acid. The product was removed by filtration, washed with cold water, and dried overnight in a desiccator. The yield of cream-colored crystals was 1.4 Gm. (50%), m.p. 118–119°.

Anal.—Calcd. for $C_{14}H_{10}O_2S_2$: S, 23.37. Found: S, 23.40.

o,o′-Bis(N-2-mercaptoethyl)diphenamide (IX).—Ethylenimine, 0.4 Gm. (0.009 mole), was added slowly to a solution of 1.1 Gm. (0.004 mole) of thiodiphenic acid (VIII) in 20 ml. of absolute ethanol with constant stirring at 0°. The reaction mixture, under a nitrogen atmosphere, was allowed to come to room temperature, and the solvent was removed *in vacuo*. The residue was crystallized from ethanol–ether to give 1.2 Gm. (82%) of yellow crystals, m.p. 129–130°. The infrared spectrum showed a well defined band at 2600 $cm.^{-1}$(−sh).

Anal.—Calcd. for $C_{18}H_{20}N_2O_2S_2$: C, 59.97; H, 5.60; N, 7.77; S, 17.79. Found: C, 59.98; H, 5.65; N, 7.85; S, 17.82.

DISCUSSION

The *N*-chloropropionamido and *N*-bromopropionamido derivatives of diphenamide, compounds V and VI, respectively, were also prepared by treating a cold methanolic solution of *o,o′*-bis(*N*-2-aminoethyl)diphenamide with ethyl 3-chloropropionate and methyl 3-bromopropionate. However, an extended reaction period at 0° was required, and the yields of the haloamides were considerably lower than when the acyl chlorides were employed.

Preliminary pharmacological studies indicate that compounds V and VI are nontoxic in doses up to 90 mg./Kg. in mice.

In the synthesis of *o,o′*-thiodiphenic acid (VIII), two minor products were also isolated. *o,o′*-Monothiodiphenic acid had a melting point of 125–126°.

Anal.—Calcd. for $C_{14}H_{10}O_3S$: C, 65.10; H, 3.90; S, 12.42. Found: C, 64.96; H, 3.79; S, 12.50.

o,o′-Diphenoyl sulfide had a melting point of 91–92°.

Anal.—Calcd. for $C_{14}H_8O_2S$: C, 69.98; H, 3.36; S, 13.34. Found: C, 69.84; H, 3.40; S, 13.42.

SUMMARY

Four new derivatives of *o,o′*-diphenamide have been synthesized to evaluate their anticarcinogenic activity: *o,o′*-bis[*N*-2-(3-chloropropionamido)ethyl]-diphenamide, *o,o′*-bis[*N*-2-(3-bromopropionamido)-ethyl]diphenamide, *o,o′*-bis(*N*-2-mesyloxyethyl)diphenamide, and *o,o′*-bis(*N*-2-mercaptoethyl)diphenamide. Two intermediate products, *o,o′*-bis(*N*-2-aminoethyl)diphenamide and *o,o′*-bis(*N*-2-hydroxyethyl)diphenamide, and two by-products, *o,o′*-monothiodiphenic acid and *o,o′*-diphenoyl sulfide, were also reported.

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