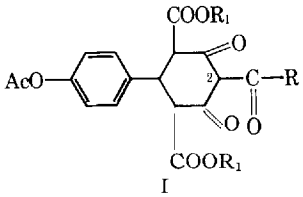
Potential Anticancer Compounds

Synthesis of 2-Carbamoyl and 2-Phenyl Carbamoyl Derivatives of 1,3-Cyclohexanedione-Type Compounds

By PHILIPPOS E. PAPADAKIS and GUY HAVEN

2-Carbamoyl and 2-phenylcarbamoyl derivatives of 1,3-cyclic diketones (I–VI) were synthesized as potential antibacterial and anticancer reagents.

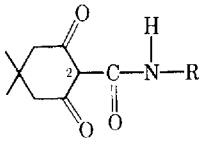
IN PREVIOUS publications (1, 2) the synthesis of 2-acyl and 2-aroyl derivatives of 1,3-cyclohexanedione-type compounds were reported. Compound I is an example where R = alkyl or aroyl and R_1 = ethyl.



I

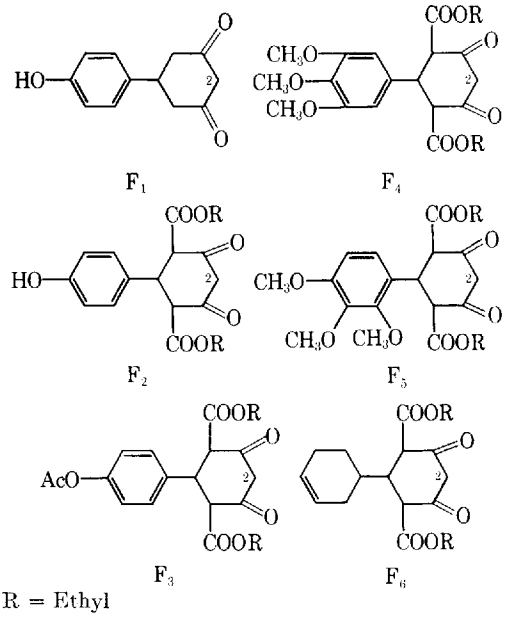
Carbon 2 in formula I has a tricarbonyl methane structure.

A Japanese team of scientists (3) found that the acylated or aroylated derivatives, at position 2 of the cyclic diketones they used, inhibited the multiplication of the Gram-positive bacteria while the carbamoyl and phenyl carbamoylated derivatives showed potency against both Gram-positive and Gram-negative bacteria, as well as a wide spectrum of antitumor activity. An example of such derivatives of the 5,5-dimethyl-1,3-cyclohexanedione (dimedone) is given by the formula (II) where R = H or C_6H_5.



II

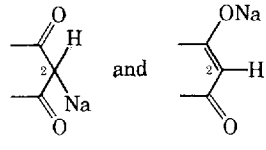
Papadakis *et al.* have synthesized derivatives of 1,3-cyclohexanedione (4–6) which have been used as intermediates for the preparation of physiologically important compounds (2, 7–10). Following are the formulas of such intermediates which have structural relationship to dimedone.



F_1 F_4 F_2 F_5 F_3 F_6

R = Ethyl

In view of the findings of Ukita *et al.* (3), it was thought advisable to prepare derivatives of each of the compounds I–VI having the —$CONH_2$ or —$CONHC_6H_5$ attached to 2-C of the cyclohexanedione ring. There are several methods by which such derivatives may be obtained but each has its own difficulties and side reactions. The phosgene (ClCOCl) reaction with the sodio derivative of each of the compounds I–VI would seem to afford a direct method for the preparation of the products desired. The method has the disadvantage that when each of the compounds F_1–F_6 is treated with NaOR, two structures result.



When the sodio derivatives are treated with phosgene and subsequently with ammonia, the following products may be obtained:

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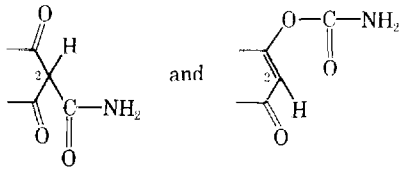
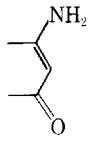
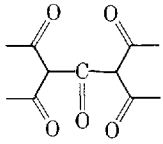


III IV

Product IV predominates. Ammonolysis of IV may render as by-products urea and

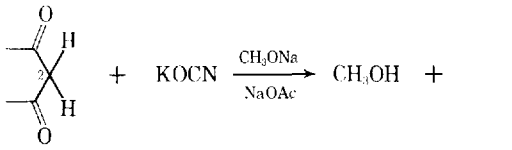


Another possibility is the formation of bis derivatives type V. When the cyclic diketone,



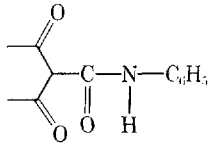
V

F_1, reacts with phosgene in the molar proportions of 2:3 and the product is treated with ammonia or aniline, compound VI is formed.



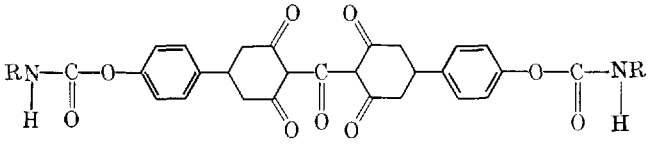
Scheme I

The 2-phenyl carbamoyl derivatives



of any of the formulas F_1–F_6 can be prepared by one of the reactions shown in Scheme II.

Similar side reactions can occur here as in the case of the preparation of the —$CONH_2$ derivative. In reaction *B* where aniline was used, there



VI

R = H or C_6H_5, respectively.

Such carbamoate or phenylcarbamoate derivatives have been isolated and analyzed (11).

There is also the possibility of polymerization between the sodioderivatives of F_1 and phosgene.

In another method each of the structures (F_1–F_6) was treated with phosgene in the presence of pyridine. Here also there is the possibility of formation of bis compounds. When any of the compounds (structures F_1–F_6) suspended in dioxane is mixed with the pyridine and the mixture, from a dropping funnel, is allowed to fall dropwise into the reaction mixture flask containing excess of a solution of 12% phosgene in benzene, the formation of the bis compound decreases.

Another method involves the reaction of any of the compounds (F_1–F_6) with potassium cyanate in the presence of $NaOCH_3$ and sodium acetate using dioxane and refluxing temperature. (Scheme I.)

is the possibility of carbanilide formation, C_6H_5-$NHCONHC_6H_5$. The method of mixing the reagents as indicated previously can adjust the relative concentrations, thereby decreasing the amount of the carbanilide formation.

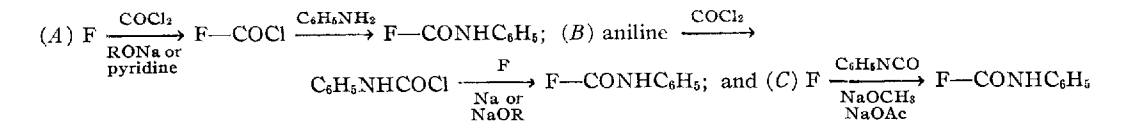
EXPERIMENTAL

In experiments *1*, *9*, and *12*, the phosgene method was used. The apparatus set up, safety precautions, and the general procedure are described in experiment *1*.

Instead of writing the long names of the compounds F_1, F_2, F_3 . . . etc., the respective letters will be used to represent the compounds. The names of the products will be given in the beginning of each experiment.

In experiments *10*, *11*, and *13*, the procedure was similar to that of experiment *1*, except that aniline was used instead of ammonia, followed by refluxing, concentrating, and processing.

Experiments *3*, *6*, *7*, and *14* were done using the phenyl isocyanate method as described.



Scheme II

1.—5 - (p - Hydroxyphenyl) - 2 - carbamoyl - 1,3-cyclohexanedione.—A mixture of 10.5 Gm. of F_1 (0.0515 mole), dioxane (30 ml.), and pyridine (8 ml.) was added slowly through a dropping funnel into a three-neck flask which contained 130 ml. of a solution of 12% phosgene in benzene. The reaction mixture flask was equipped with a dropping funnel, a condenser, and a magnetic stirrer. The top of the reflux condenser was connected with a flask containing NaOH to trap any $COCl_2$ vapor. All the apparatus was set up under the hood.

The phosgene–benzene solution was kept cold during the addition of the diketone–pyridine–dioxane mixture. The reaction mixture was allowed to stand at room temperature under the hood for 2 days, then it was refluxed for 2 hr. The flask containing the NaOH was connected to the water aspirator and controlled reduced pressure was applied to remove any unreacted phosgene. Arrangements were made for distillation. The heating was continued till the temperature reached 98°. After cooling, 30 ml. of dioxane was added to the mixture, and ammonia gas was bubbled through it for 2 hr. The mixture was allowed to stand overnight. The precipitate formed was filtered. The filtrate was concentrated to a red-orange syrup which was stirred with ether 3 successive times. The ether was decanted each time. The light tan residue was dried on porous tile. The combined solids (9 Gm.) were boiled with a limited amount of absolute alcohol and filtered hot. On cooling, the filtrate rendered crystals which were washed with distilled water and dried on porous tile, m.p. 167°–176°.

Anal.—Calcd. for $C_{13}H_{13}NO_4 \cdot H_2O$: C, 58.87; H, 5.64; N, 5.28. Found: C, 59.21; H, 5.30; N, 5.40.

The material above was dried at 100° and 1.5 mm., m.p. 183°.

Anal.—Calcd. for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.26; N, 5.66. Found: C, 63.27; H, 5.65; N, 5.25.

The material which was not readily soluble in the hot alcohol, m.p. 185°, corresponds to the formula $C_{14}H_{14}N_2O_5$.

2.—5-(p-O-Carbamoate phenyl)-2-carbamoyl-1,3-cyclohexanedione.—*Anal.*—Calcd. for $C_{14}H_{14}N_2O_5$: C, 57.93; H, 4.82. Found: C, 58.02; H, 4.70.

3.—2 - Phenylcarbamoyl - 5 - (p - O - phenylcarbamoate phenyl) - 1,3 - cyclohexanedione.—F_1, 7.28 Gm. (0.035 mole), 0.805 Gm. of sodium dissolved in methanol, and 30 ml. of dioxane were mixed in a three-neck flask equipped with a reflux condenser and magnetic stirrer. After refluxing for 20 min., the apparatus was arranged for distillation of the methanol. The distillation was continued until the temperature reached 90°. Twenty milliliters of dioxane was added and 4.2 ml. of phenyl isocyanate. After 5 hr. of refluxing, the dioxane was distilled off under reduced pressure using a water bath. The residue was cooled and then washed with ether. The ether was decanted and the residue was stirred with water. The solution was acidified and the precipitate which formed was filtered and recrystallized from ethanol. It sinters at 193°. It turns to syrupy droplets at 198° and to a red-black melt at 223°–229°. Recrystallized from acetone, m.p. 209°–213°.

Anal.—Calcd. for $C_{26}H_{22}N_2O_5$: C, 70.57; H, 4.97; N, 6.33. Found: C, 70.16, 70.31, 70.53; H, 4.73, 5.22, 5.10; N, 6.32.

4.—5 - (p - Hydroxyphenyl) - 2 - carbamoyl - 4,6-dicarbobutoxy - 1,3 - cyclohexanedione.—F_2 (10.4 Gm.), sodium (0.92 Gm.) dissolved in butanol, urea (3.6 Gm.), and butanol (50 ml.) were refluxed 4 hr. Some urea sublimed and was deposited in the inner wall of the reflux condenser. Some ammonia was also forming, as shown when a piece of litmus paper held on the top of the condenser turned blue. Some methyl alcohol was added to lower the boiling temperature to 95° and prevent the subliming of urea. The materials dissolved in the beginning. Later a fine precipitate formed which changed from a greenish to a brown color.

After 4 hr. of refluxing, most of the butanol was distilled under reduced pressure. Water was added, and the mixture was acidified. The precipitate was stirred with cold alcohol and filtered. From the filtrate, after evaporation of the alcohol, the material obtained melted at 80°–85°, then resolidified, and remelted at 175°.

Anal.—Calcd. for $C_{13}H_{29}O_8N \cdot {}^1/_2H_2O$: C, 60.52; H, 6.57. Found: C, 60.08; H, 6.57.

5.—5 - (p - Hydroxyphenyl) - 1 - amino - 2-carbamoyl - 4,6 - dicarbethoxy - 1 - cyclohexenone-3.—F_2, 10.4 Gm. (0.03 mole), urea, 3.6 Gm. (0.06 mole), and dimethylformamide were refluxed 8 hr. After the refluxing, most of the solvent was evaporated under reduced pressure. Water was added. The mixture was stirred and filtered. The residue had a melting point of 187°–190°. The product shows blue fluorescence under ultraviolet light.

Anal.—Calcd. for $2(C_{19}H_{22}O_7) \cdot H_2O$: C, 59.84; H, 5.51; N, 7.34. Found: C, 59.39; H, 5.33; N, 7.40.

6.—2 - Phenylcarbamoyl - 5 - (p - hydroxyphenyl) - 4,6 - dicarbethoxy - 1,3 - cyclohexanedione.—F_2, 10.4 Gm. (0.03 mole), NaOAc, 2.46 Gm., C_6H_5NCO, 7.14 Gm. (0.06 mole), and $NaOCH_3$, 3.18 Gm. (0.03 mole) were refluxed in dioxane medium for 8 hr. After that, the mixture was acidified and the precipitate was filtered off, washed with distilled water and with alcohol, m.p. 226°. The filtrate was concentrated by evaporation under reduced pressure and the residue recrystallized from ethanol, m.p. 195°.

Anal.—Calcd. for $C_{25}H_{25}NO_8 \cdot H_2O$: C, 61.84; H, 5.60. Found: C, 61.54; H, 5.75.

7.—5 - (p - Acetoxyphenyl) - 2 - phenyl carbamoyl-4,6 - dicarbethoxy - 1,3 - cyclohexanedione.—F_3, 3.9 Gm. (0.01 mole), C_6H_5NCO (1.2 Gm.), NaOAc anhydrous (1 Gm.), and 30 ml. of dioxane were mixed. After 4 hr. of refluxing, most of the dioxane

was distilled under reduced pressure. The residue was stirred in water 3 hr. and then filtered. The crystals were dried and then washed with ether three times, 223° dec. Product was almost white (little pale yellow).

Anal.—Calcd. for $C_{27}H_{27}O_9 \cdot H_2O$: C, 61.48; H, 5.54. Found: C, 61.91; H, 5.05.

Anal.—Calcd. for $C_{25}H_{21}O_8 \cdot H_2O$: C, 62.37; H, 5.00. Found: C, 61.91; H, 5.05.

8.—5 - (p - Acetoxyphenyl) - 2 - carbamoyl - 4,6-dicarbethoxy - 1,3 - cyclohexanedione.—A mixture of F_3 (11.7 Gm.), KOCN (2.43 Gm.), $NaOCH_3$ (1.62 Gm.), NaOAc (2.46 Gm.), and 50 ml. of *N*-dimethylformamide was refluxed for 5 hr. The solution became greenish, then red-orange, and orange-brown. Chromatographic paper is stained orange. Under the influence of U.V. it shows strong greenish cream fluorescence. The calculated amount of hydrochloric acid was added to react with the KOCN, sodium methoxide, and sodium acetate. The solution was evaporated under reduced pressure and the residue stirred with ice water. The insoluble part was washed with ether and recrystallized from methanol, m.p. 193°.

Anal.—Calcd. for $C_{24}H_{28}N_2O_2$: C, 56.91; H, 5.57. Found: C, 56.50; H, 5.57.

The analytical results suggest that the amide of the carbamoyl of the title compound may have reacted with the dimethylformamide as follows:

$$F\text{—}CONH_2 + O{=}C(H)\text{—}N(CH_3)_2 \rightarrow RCON{=}CH\text{—}N(CH_3)_2$$

9.—5 - (p - Acetoxyphenyl) - 2 - carbamoyl - 1,4-carbolactone - 6 - carbethoxy - 1 - cyclohexenone-3.—An attempt was made to make the compound (experiment *8*) by the phosgene method used in experiment *1*, using as starting material F_3 instead of F_1. The product begins to gum at 210° and tars at 280°. It is insoluble in ether, dioxane, cold alcohol, and water.

Anal.—Calcd. for $[C_{21}H_{23}NO_9 \cdot H_2O] \cdot C_2H_5OH$: C, 56.29; H, 4.72. Found: C, 56.50; H, 5.11.

10.—5 - (3′,4′,5′ - Trimethoxyphenyl) - 2 - phenyl carbamoyl - 4,6 - dicarbethoxy - 1,3 - cyclohexanedione.—F_5, 4.22 Gm. (0.01 mole) (6), dioxane (30 ml.), and pyridine (1 ml.) were added slowly through a dropping funnel into a three-neck flask containing 15 ml. of a 12% solution of $COCl_2$ in benzene. The apparatus and procedure were similar to experiment *1* with the exception that 1 ml. of aniline instead of ammonia was used. After refluxing for several hours, the dioxane was evaporated under reduced pressure. The residue was stirred with distilled water and acidified, filtered, and dried, and then it was washed with ether, m.p. 193°–195°, as aniline hydrochloride melts at 198°, mixed melting points were taken, 150°–155°.

Anal.—Calcd. for $C_{28}H_{31}NO_{10}$: C, 62.10; H, 5.73. Found: C, 62.58; H, 5.48.

11.—5 - (3′,4′,5′ - Trimethoxyphenyl) - 1 - iminophenyl - 2 - phenylcarbamoyl - 4,6 - dicarbethoxy-1,3-cyclohexanedione.—Experiment *10* was repeated with the difference that excess aniline was used. A material was obtained which decomposed at 122°.

Anal.—Calcd. for $C_{34}H_{36}N_2O_9$: C, 66.88; H, 6.23. Found: C, 67.11; H, 5.82.

The analytical data correspond to the title compound.

12.—5 - (4′ - Cyclohexene) - 1 - amino - 2 - carbamoyl - 4,6 - dicarbethoxy - 1 - cyclohexenone - 3.—A mixture of F_6, 11.2 Gm. (0.033 mole), dioxane (50 ml.), and pyridine (3 ml.) was added slowly through a dropping funnel into a three-neck flask which contained 70 ml. of a solution of 12% phosgene in benzene. The apparatus set up, safety precautions, and the procedure were described in experiment *1*. After the ammonia treatment, the mixture was allowed to stand overnight at room temperature. The precipitate formed was filtered and then heated with alcohol. The part that did not dissolve was washed with ether and dried on porous tile. The material had a light tan color, m.p. 150°, clear at 152°.

Anal.—Calcd. for $C_{19}H_{26}N_2O_6 \cdot {}^1/_2\ H_2O$: C, 58.91; H, 6.99. Found: C, 58.70, 58.90; H, 7.29, 7.25.

13.—5 - (4′ - Cyclohexene) - 2 - phenylcarbamoyl-4, 1 - carbolactone - 6 - carbethoxy - 1 - cyclohexenone-3.—F_6, 3.35 Gm. (0.01 mole) in dioxane, was mixed with 1 ml. of pyridine. The mixture was added dropwise through a dropping funnel into a three-neck flask containing 15 ml. of a 12% solution of phosgene in benzene. The apparatus set-up, safety precautions, and procedure were similar to experiment *1*, except that after the distillation of any unreacted phosgene, aniline instead of ammonia was used. To the reaction mixture 1 ml. of aniline in 24 ml. of dioxane was added dropwise with stirring.

The mixture was refluxed for 1 hr., then concentrated. The precipitate formed was filtered then stirred with water to get rid of the pyridinium chloride. The residue was dried, washed with ether, and recrystallized from acetone, m.p. 230°.

14.—In another experiment equimolar quantities of F_6 and CH_3ONa in dioxane medium were refluxed for 0.5 hr. The apparatus was arranged for distillation. Part of the solvent was allowed to distil until the temperature reached 100°. To the remaining mixture, equimolar quantities of phenyl isocyanate and anhydrous sodium acetate were added, and the mixture was refluxed for 2 hr. The precipitate formed was washed with water, filtered, dried, and then washed with ether. The ether was decanted and the residue recrystallized from acetone, m.p. 228°–229°; m.p. with carbanilide 208°.

Anal.—Calcd. for $C_{23}H_{24}NO_6 \cdot 2H_2O$: C, 61.88; H, 6.27; N, 3.13. Found: C, 62.04; H, 5.86; N, 2.90.

The material dried at 100° and 1.5 mm. gave the following analysis.

Anal.—Calcd. for $C_{23}H_{24}NO_6$: C, 68.03; H, 6.57. Found: C, 68.25; H, 6.56.

15.—Diethyl - (3 - cyclohexenal) - malonate.—3-Cyclohexene-1-carboxaldehyde, 218 Gm. (2 moles), 320 Gm. of diethylmalonate (2 moles), and 15 ml. of piperidine were mixed and refluxed 24 hr. at 105°. The mixture became orange in color. Fractional distillation was applied under reduced pressure. The fraction boiling at 132°–138° and 0.7 mm. was taken as representing the title compound and was used for the next step.

16.—5 - (4′ - Cyclohexene) - 4,6 - dicarbethoxy 1,3 - cyclohexanedione.—Sodium (7.8 Gm.) dissolved in absolute ethanol was added with stirring to 50 ml. of ethyl acetoacetate. After 15 min. of refluxing it was poured into a 1000-ml. flask contain-

ing 100 Gm. of diethyl-(3-cyclohexenal)-malonate. The mixture was refluxed for 6 hr. The color of the solution turned red. Distillation of the solvents under reduced pressure followed using a flash evaporator. The residue was dissolved in cold distilled water. The solution was extracted twice with ether. The aqueous layer was adjusted to pH 7 and then it was extracted with ether once more. The water layer was acidified with 3 *N* hydrochloric acid. White crystals resulted which were filtered, washed with distilled water, filtered, and then washed with a 50:50 mixture of ether–petroleum ether, m.p. 111°–113°. Recrystallized 4 times from absolute alcohol, m.p. 133°–137°.

Anal.—Calcd. for $C_{18}H_{24}O_6$: C, 64.26; H, 7.19. Found: C, 64.45; H, 7.32; C, 64.45; H, 7.19.

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Comparative Study of the Alternating and Direct Current Polarography of Several Δ^4-3-Ketosteroids

By JAMES L. SPAHR and ADELBERT M. KNEVEL

A study was undertaken to compare alternating current polarography with direct current polarography as a method of analysis of testosterone, methyltestosterone, and progesterone. A solution consisting of 50 per cent ethanol, buffer (pH 1.3), and tetrabutylammonium iodide was used as the sample medium. Results showed that the lowest practical concentrations of detection for both a.c. and d.c. polarography was $3.3 \times 10^{-5}M$. However, a.c. polarography gave greater precision than did the d.c. method.

A STUDY OF the analysis of Δ^4-3-ketosteroids by direct current (d.c.) polarography has been reported by several groups of workers (1–3). In the study conducted by Kabasakalian and McGlotten (3), it was reported that the diffusion current of testosterone and other related Δ^4-3-ketosteroids was directly proportional to the concentration in the range of 2×10^{-4} to $1 \times 10^{-2}M$. At low concentrations, however, deviations from linearity were observed in some cases. These workers pointed out that the deviations may have been due to the method of measuring the diffusion current rather than a change in diffusion properties, because the diffusion current plateau at low concentration was too steep. This explanation seems reasonable since ill-defined polarographic waves are not uncommon with ketones. One factor contributing to this poor definition may be that ketone half-wave potentials occur very close to the discharge potentials of the buffer components. This effect often makes it difficult to separate ketone diffusion current from buffer discharge current. Alternating current (a.c.) polarography offers the advantage of producing polarograms in which the reduction waves of the ketone and buffer components are often sufficiently separated so that diffusion currents can be measured more accurately. Furthermore, this technique is often more sensitive to organic compounds than is d.c. polarography. The objective of this study was to compare a.c. polarography with d.c. polarography as a method of analysis for several different Δ^4-3-ketosteroids.

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EXPERIMENTAL

Apparatus.—The dropping mercury electrode capillary used in this study had a length of 9.3 cm. Under a pressure of 26.5 cm. of mercury and with an open circuit, the drop time was 4.86 sec. and *m* was 1.13 mg. $sec.^{-1}$. These characteristics were determined at 25° with the mercury dropping into 50% ethanol which was 0.1 *M* in tetrabutylammonium iodide.

The electrolysis cell was a tube 7 cm. in length with an inside diameter of 2.1 cm. The saturated calomel reference used throughout this work was contained in a Hildebrand half-cell. Junction be-