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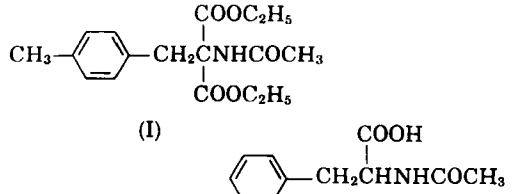
Research Articles

Methyl-Substituted Tyrosines and Related Compounds as Potential Anticancer Agents

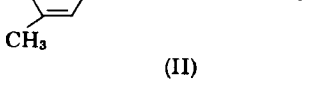
By EUGENE C. JORGENSEN and ROBERT A. WILEY†

3,5-Dimethyl-DL-tyrosine, 3-methyl-DL-tyrosine, 3-methyl-5-iodo-DL-tyrosine, as well as synthetic intermediates and derivatives of these, have been prepared for study of anticancer activity. Preliminary tests on two members of the series against experimental tumors in mice showed a slight inhibition of tumor growth.

THE SYNTHESIS and antitumor activity of a number of derivatives of phenylalanine, N-acetylphenylalanine, and diethyl α-acetamido-α-benzylmalonate have been reported (1, 2). The most active members of this series were diethyl α-acetamido-α-(4-methylbenzyl) malonate (I) and N-acetyl-3-methyl-DL-phenylalanine (II). Studies relative to the synthesis of analogs of thy-



(I)



(II)

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TABLE I.—SCREENING TEST FOR ANTICANCER ACTIVITY AGAINST ASCITES TUMORS IN MICE

Compound	Dose mg. per Kg. Body Weight	Gardner (ED) Lymphosarcoma TPCV,[a] %	Gardner (ED) Lymphosarcoma Survival,[b] days	Erhlich (E2) Carcinoma TPCV,[a] %	Erhlich (E2) Carcinoma Survival,[b] days	L4946 (C1) Leukemia TPCV,[a] %	L4946 (C1) Leukemia Survival,[b] days
V	500	126	+2.0	82	...	48	−1.5
VII	500	137	+2.0	65	...	77	−1.5

[a] TPCV = (Total packed cell volume treated/total packed cell volume controls). [b] Survival = (Median survival treated)-(median survival controls).

roxine involved the preparation of methyl-substituted derivatives of DL-tyrosine, and synthetic intermediates closely related to I. From these, a series of derivatives has been prepared for study as antitumor agents.

The chloromethylation procedure of Bielig (3) was adapted in the preparation of 3,5-dimethyl-4-methoxybenzyl chloride (III) and of 3-methyl-4-methoxybenzyl chloride (IV) from the corresponding methyl-substituted anisoles. 3,5-Dimethyl-4-methoxybenzyl chloride was condensed with ethyl acetamidocyanoacetate, and the resulting α-substituted cyanoacetic ester (V) converted into 3,5-dimethyl-DL-tyrosine (VI) by prolonged heating with hydriodic acid. Following the completion of this work, Dibbo, *et al.* (4), have reported a similar synthesis of 3,5-dimethyl-DL-tyrosine proceeding through the α-substituted diethyl acetamidomalonate (VII), which was also prepared in this study. This intermediate was used to prepare derivatives retaining the O-methyl group by selective hydrolysis to the acetamidomalonic acid (VIII), decarboxylation to the acetamido acid (IX), and further hydrolysis to form 3,5-dimethyl-4-methoxy-DL-phenylalanine (X).

3-Methyl-DL-tyrosine (XII) was obtained by the condensation of 3-methyl-4-methoxybenzyl chloride with diethyl acetamidomalonate, followed by hydrolysis of the ester (XI) with hydriodic acid. Iodination of XII in aqueous ethylamine yielded 3-methyl-5-iodo-DL-tyrosine (XIII).

The amino acids (VI, XII, XIII) were converted into their N-acetyl (XIV–XVI) and N-acetyl ethyl ester (XVII–XIX) derivatives.

Two of the intermediates, ethyl α-acetamido-α-cyano-β-(3,5-dimethyl-4-methoxyphenyl)-propionate (V) and diethyl α-acetamido-α-(3,5-dimethyl-4-methoxybenzyl)malonate (VII) were submitted for preliminary antitumor screening against lymphosarcoma, carcinoma, and leukemia tumors in mice[1] by the method of Costa, Blumenthal, and Greenberg (5). As shown in Table I, the compounds were inactive against the lymphosarcoma, but showed slight activity against the carcinoma (18 and 35% inhibition) and leukemia (52 and 23% inhibition). The remaining compounds herein reported will be submitted for antitumor screening.

EXPERIMENTAL[2]

3,5-Dimethyl-4-methoxybenzyl Chloride (III).—A mixture of 2,6-dimethylanisole (71 Gm., 0.5 mole), glacial acetic acid (500 ml.), and paraformaldehyde (20 Gm., 0.65 mole) was stirred and cooled in an ice bath until the temperature reached 10°. Concentrated hydrochloric acid (60 ml.) was added, and hydrogen chloride was passed into the stirred mixture for 11 hours. The temperature was held below 10° for the first 2 hours and at room temperature thereafter. The mixture was poured into 2 L. of ice water, and extracted with chloroform. The chloroform extract was washed successively with water, 1 *N* sodium bicarbonate solution, and water, and dried over anhydrous sodium sulfate. The chloroform was removed under reduced pressure, and the residual oil flash-distilled under nitrogen at reduced pressure. There was obtained 66.4 Gm. (68.6%) of an almost colorless liquid, n_D^{20} 1.5294, b.p. 124–128° (4 mm.) [Lit. (4), n_D^{20} 1.5375, b.p. 83–85° (1 mm.)].

Anal.—Calcd. for $C_{10}H_{13}ClO$: Cl, 19.25. Found: Cl, 18.89.

3-Methyl-4-methoxybenzyl Chloride (IV).—Prepared[3] from 2-methylanisole as described above, b.p. 125° (5 mm.), n_D^{20} 1.539.

Ethyl α-Acetamido-α-cyano-β-(3,5-dimethyl-4-methoxyphenyl)propionate (V).—To sodium (0.58 Gm., 0.025 mole) in absolute ethanol (10 ml.) was added in one portion ethyl acetamidocyanoacetate (4.25 Gm., 0.025 mole). 3,5-Dimethyl-4-methoxybenzyl chloride (III, 4.61 Gm., 0.025 mole) was added dropwise over 20 minutes to the stirred solution. The reaction mixture was heated under reflux for 1 hour, then poured into ice water. The yellow oil which separated solidified on standing to yield 6.99 Gm. (88%); recrystallization from methanol gave colorless plates, m.p. 139–140°.

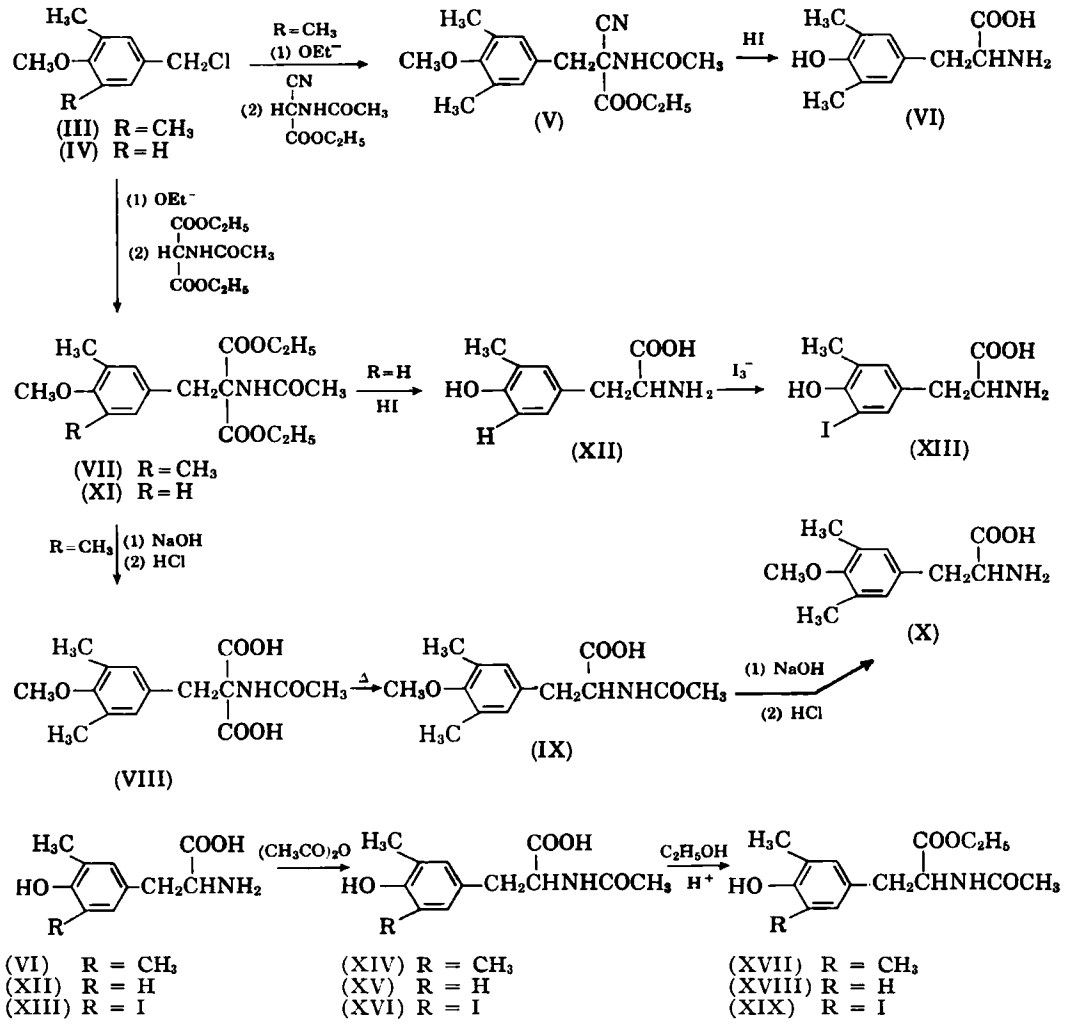
Anal.—Calcd. for $C_{17}H_{22}N_2O_4$: N, 8.81. Found: N, 8.66.

3,5-Dimethyl-DL-tyrosine (VI).—The preceding ester (V, 5.0 Gm., 15.4 mmoles) was heated under reflux for 4 hours with a mixture of glacial acetic acid (25 ml.) and 48% hydriodic acid (25 ml.). The reaction mixture was evaporated to dryness

[1] The authors are indebted to Dr. D. M. Greenberg for the biological results.

[2] Melting points obtained on a Fisher-Johns melting point apparatus and are uncorrected. Microanalyses by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley.

[3] Prepared by Mr. Menlo Kawakami, to whom we express our gratitude.



in vacuo, and the light pink residue dissolved in hot water (5 ml.). Concentrated sodium acetate solution was added to pH 5; after overnight refrigeration, 3.03 Gm. (92%) of yellowish powder was collected. An analytical sample was prepared by iso-electric precipitation at pH 5, m.p. 240–243° (decompn.) [Lit. (4), 242–248° (decompn.)].

Anal.—Calcd. for $C_{11}H_{15}NO_3$: C, 63.16; H, 7.23. Found: C, 62.88; H, 7.13.

Ethyl α-Acetamido-α-(3,5-dimethyl-4-methoxybenzyl)malonate (VII).—To sodium (1.15 Gm., 0.05 mole) dissolved in absolute ethanol (125 ml.) was added in one portion ethyl acetamidomalonate (10.85 Gm., 0.05 mole). 3,5-Dimethyl-4-methoxybenzyl chloride (III, 9.22 Gm., 0.05 mole) was added dropwise over 20 minutes to the stirred solution. The reaction mixture was heated under reflux for 1 hour, then poured into ice water. The oil which separated soon solidified, affording 11.6 Gm. (64%) of colorless prisms. Recrystallized from hexane it had m.p. 94–95° [Lit. (4), 97–99°].

Anal.—Calcd. for $C_{19}H_{27}NO_6$: C, 62.44; H, 7.45. Found: C, 62.59; H, 7.39.

Ethyl α-Acetamido-α-(3-methyl-4-methoxybenzyl)malonate (XI).—Prepared[3] from 3-methyl-4-methoxybenzyl chloride (IV) as described above in 83.5% yield, m.p. 106–107°. An unstable polymorphic form melting at 113° was also observed, which reverted to the lower melting, more stable form on standing.

Anal.—Calcd. for $C_{18}H_{25}NO_6$: C, 61.51; H, 7.17. Found: C, 61.77; H, 6.99.

α - Acetamido - α - (3,5 - dimethyl - 4 - methoxybenzyl)malonic Acid (VIII).—The malonate ester (VII, 2.0 Gm., 5.47 mmoles) was dissolved in a solution of sodium hydroxide (5 Gm.) in 50% ethanol (50 ml.) and stirred at room temperature for 24 hours. Most of the alcohol was evaporated *in vacuo*, and the unreacted ester removed from the aqueous residue by extraction with chloroform. The solution was cooled to 10° and acidified with concentrated hydrochloric acid, whereupon 1.0 Gm. (59%) of white precipitate separated. This was crystallized from acetone-water, and from acetone-hexane, m.p. 143–144°.

Anal.—Calcd. for $C_{15}H_{19}NO_6$: C, 58.28; H, 6.19. Found: C, 58.49; H, 6.26.

N - Acetyl - 3,5-dimethyl - 4 - methoxy - DL-

phenylalanine (IX).—The preceding acetamido acid (VIII, 0.8 Gm., 2.6 mmoles) was heated at a bath temperature of 180° for 30 minutes. The resulting yellow oil was taken up in chloroform, extracted with 2% sodium hydroxide, the aqueous extract acidified, and extracted with ether. The ether solution was dried over anhydrous sodium sulfate, filtered, and allowed to evaporate in the air, affording 0.3 Gm. (44%) of colorless prisms. Crystallized from ethanol-water, it had m.p. 151.5–152°.

Anal.—Calcd. for $C_{14}H_{19}NO_4$: C, 63.39; H, 7.22. Found: C, 63.31; H, 7.20.

3,5-Dimethyl-4-methoxy-DL-phenylalanine (X).—The preceding N-acetyl derivative (IX, 3.4 Gm., 12.8 mmoles) was heated under reflux with 100 ml. of 10% aqueous sodium hydroxide for 24 hours. Acidification with concentrated hydrochloric acid resulted in a white inorganic precipitate which was removed by filtration, and the filtrate evaporated to dryness *in vacuo*. The residue was extracted with 250 ml. of boiling absolute ethanol which, on evaporation, yielded 0.7 Gm. (25%), m.p. 250° (decompn.). Purification was effected by isoelectric precipitation at pH 5.

Anal.—Calcd. for $C_{12}H_{17}NO_3$: C, 64.50; H, 7.67. Found: C, 64.22; H, 7.76.

3-Methyl-DL-tyrosine (XII).—The acetamidomalonic ester (XI, 25 Gm., 0.071 mole), 47% hydriodic acid (100 ml.), and glacial acetic acid (100 ml.) were heated under reflux for 7 hours. Additional hydriodic acid (60 ml.) was added and heating was continued overnight. The acids were removed by distillation *in vacuo*, the residue taken up in water, and the pH of the solution adjusted to 5 with 40% aqueous sodium hydroxide, affording 21.6 Gm. of white solid. One isoelectric precipitation at pH 5 yielded 12 Gm. (86%) of white amorphous powder, m.p. 285° (decompn.) [Lit. (6), 276°].

3-Methyl-5-iodo-DL-tyrosine (XIII).—To a well-stirred solution of 3-methyl-DL-tyrosine (XII, 10 Gm., 0.051 mole) in 33% aqueous ethylamine (100 ml.), a solution of iodine (15.5 Gm., 0.061 mole) and potassium iodide (20 Gm.) in water (100 ml.) was added dropwise at room temperature. After the addition was complete, stirring was continued for 30 minutes, excess iodine was reduced with aqueous sodium bisulfite, and the pH of the solution was adjusted to 5 with acetic acid. The resulting precipitate was removed by filtration and purified by isoelectric precipitation at pH 5, yielding 12.2 Gm. (74.5%), m.p. 213–214° (decompn.). The compound was not obtained analytically pure.

N-Acetyl-3,5-dimethyl-DL-tyrosine (XIV).—To 3,5-Dimethyl-DL-tyrosine (VI, 1.5 Gm., 7.18 mmoles) dissolved in 2 *N* sodium hydroxide (31 ml.) and maintained at 5–10°, acetic anhydride (3.45 ml.) was added over a period of 1.5 hours. After standing overnight at room temperature, the solution was cooled to 10° and a solution of sodium hydroxide (1.49 Gm.) in water (3.8 ml.) was added, followed by ethanol (18 ml.), and concentrated hydrochloric acid to pH 1.5. Upon partial evaporation, 1.42 Gm. of brownish crystals was deposited. Crystallization from ethanol-water yielded 1.0 Gm. (55%) of colorless crystals, m.p. 182–183° [Lit. (4), 183–185°].

Anal.—Calcd. for $C_{12}H_{17}NO_4$: C, 62.12; H, 6.82. Found: C, 62.51; H, 7.19.

N-Acetyl-3-methyl-DL-tyrosine (XV).—3-Methyl-DL-tyrosine (XII) was acetylated as described above. The resulting oil resisted crystallization, but was identified by conversion to the N-acetyl ethyl ester (XVIII).

N-Acetyl-3-methyl-5-iodo-DL-tyrosine (XVI).—The N-acetyl derivative was prepared in 80% yield from 3-methyl-5-iodo-DL-tyrosine (XIII) as previously described, m.p. 180–181°.

Anal.—Calcd. for $C_{12}H_{14}INO_4$: C, 39.68; H, 3.89. Found: C, 39.87; H, 4.03.

N-Acetyl-3,5-dimethyl-DL-tyrosine Ethyl Ester (XVII).—N-Acetyl-3,5-dimethyl-DL-tyrosine (XIV, 4.7 Gm., 18.7 mmoles), *p*-toluenesulfonic acid (0.6 Gm.), absolute ethanol (6 ml.), and chloroform (60 ml.) were heated together under reflux for 8 hours, during which the azeotropic mixture of water, ethanol, and chloroform was removed by distillation. Additional chloroform was added as needed to maintain a minimum volume of 60 ml. After 4 hours an additional 5 ml. of absolute ethanol was added. The reaction mixture was washed with 5% aqueous sodium bicarbonate and with water, dried over anhydrous calcium chloride, and the chloroform removed by distillation under reduced pressure. The residual brown oil solidified upon standing, and was crystallized from ethanol-water and from ethyl acetate-hexane, yielding 3.8 Gm. (72%), m.p. 96–97° [Lit. (4), 101–102°].

Anal.—Calcd. for $C_{15}H_{21}NO_4$: C, 64.48; H, 7.58. Found: C, 64.20; H, 7.33.

N-Acetyl-3-methyl-DL-tyrosine Ethyl Ester (XVIII).—The oily N-acetyl derivative (XV) was esterified as described above, yielding a crystalline compound, m.p. 133–134°. The overall yield from 3-methyl-DL-tyrosine was 56%.

Anal.—Calcd. for $C_{14}H_{19}NO_4$: C, 63.38; H, 7.22. Found: C, 63.57; H, 7.11.

N-Acetyl-3-methyl-5-iodo-DL-tyrosine Ethyl Ester (XIX).—The N-acetyl derivative (XVI) was esterified as described above, yielding (66%) a crystalline compound, m.p. 126–127°.

Anal.—Calcd. for $C_{14}H_{18}INO_4$: C, 42.98; H, 4.64. Found: C, 43.03; H, 4.50.

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