Synthesis and Cytotoxic Activity of Latentiated Derivatives of 3-Methyleneoxindole

M. J. KORNET ˣ, A. P. THIO, and J. H. THORSTENSON

Abstract □ Several latentiated derivatives of 3-methyleneoxindole were synthesized and examined for cytotoxic activity. The latentiated forms are represented by three general types which differ in leaving and cleaved groups. All are expected to be converted into 3-methyleneoxindole by bioactivation processes normally occurring in the cell. Two compounds were tested against L-1210 lymphoid leukemia, and six were tested against P-388 lymphocytic leukemia; all were inactive.

Keyphrases □ 3-Methyleneoxindole—latentiated derivatives synthesized and evaluated for cytotoxic activity □ Oxindoles, substituted—latentiated derivatives synthesized and evaluated for cytotoxic activity □ Cytotoxic activity—evaluated in latentiated derivatives of 3-methyleneoxindole □ Structure–activity relationships—latentiated derivatives of 3-methyleneoxindole evaluated for cytotoxic activity

Beneficial effects were reported when several cancer patients were treated with 3-indoleacetic acid for extended periods (1). Other studies found that certain esters of 3-indoleacetic acid prolonged the survival time of white mice (2) implanted with Ehrlich ascites carcinoma. The similarity of the effect on cell growth of 3-indoleacetic acid and 3-methyleneoxindole (I) (3) and the demonstrated oxidative conversion of 3-indoleacetic acid into I (4) strongly suggest that I may be the actual pharmacological agent in the action of 3-indoleacetic acid on cancer growth.

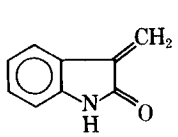
BACKGROUND

Compound I was first synthesized in 1964 (5) and is unstable. It possesses an activated ethylenic double bond and is very reactive toward the sulfhydryl group. It reacts smoothly with thiophenol (5), glutathione, and other biologically important thiol compounds (6) to give stable adducts. A sulfhydryl reagent such as I may exhibit selective cytotoxicity toward cancer cells, not only because rapidly proliferating cells reportedly expose more sulfhydryl groups (7) but also because I–sulfhydryl adducts should be more resistant toward dissociation (6) in the more acidic tumor cells (8), resulting in a slower rate of detoxification of I (9).

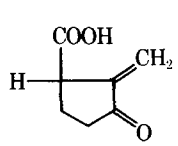
Finally, I bears a marked similarity to a number of anticancer substances including the antibiotic enzyme inhibitor sarkomycin (II) (10–12) and the antiascites substance primocarcin (III) (13, 14).

The pronounced instability and reactivity of this compound and the marked concentration dependency of its biological action (3, 15) make it impractical to apply I itself as a drug. This report is concerned with the synthesis and evaluation of latentiated forms of I, which can be converted into the active I by processes known to occur *in vivo*, *e.g.*, hydrolysis, oxidation, and reduction (16).

Immediate precursors of I that may be expected to undergo spontaneous conversion into the active compound can be represented by the three general types, A, B, and C (17), illustrated in Structures I*a*, I*b*, and I*c*, respectively. In these structures, X represents leaving groups (*e.g.*, halogens, hydroxyl, acyloxy, and amino), while Y stands for cleaved groups capable of being cleaved off from the electron pair that binds them to the rest of the molecule (*e.g.*, carboxylate and immomium). In most cases, these immediate precursors are too unstable to be useful as drugs and need to be latentiated.



I



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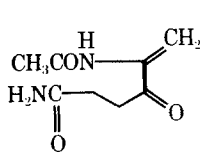

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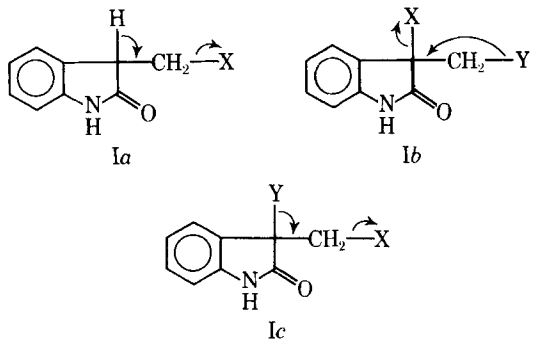


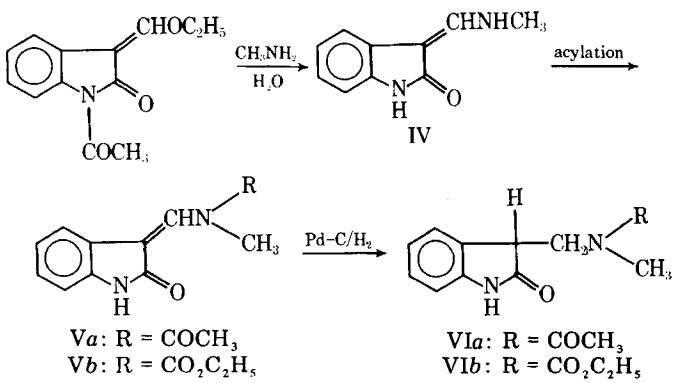
Ia Ib Ic

RESULTS AND DISCUSSION

Synthesis—Two examples of compounds of Type A were prepared (VI*a* and VI*b*, Scheme I). Compound VI*a* was obtained in four steps from oxindole (18, 19). Compound VI*b* was analogously prepared, except that acylation in the next to the last step involved ethyl chloroformate.

Three latentiated 3-methyleneoxindole derivatives of Type B were prepared. Ethyl 2,3-dioxindolyl-3-acetate (VII) (20) was synthesized from isatin and ethyl bromoacetate. Compound VII is a close relative of 3-bromo-3-oxindoleacetic acid (VIII); the latter is transformed into I almost immediately by dissolving it in water (5). For this reason, VIII (21) also was prepared for biological evaluation.

In addition, the preparation of the spirolactone X (Scheme II), which represents a third Type B compound, was attempted. 5-Bromo-2,3-dioxindole-3-butyric lactone (IX) was obtained by the *N*-bromosuccinimide oxidation of 4-indolebutyric acid according to a previous method

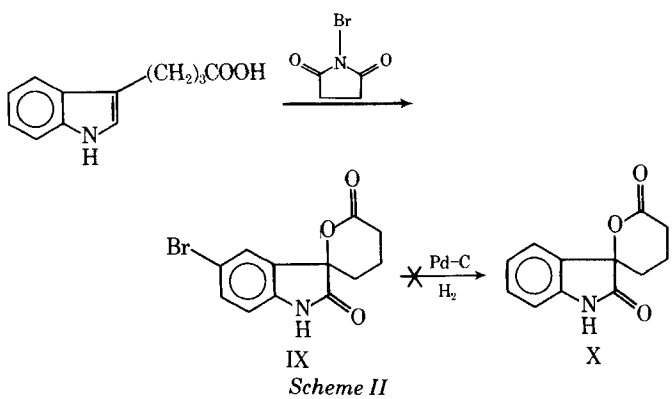


IV

Va: R = $COCH_3$
Vb: R = $CO_2C_2H_5$

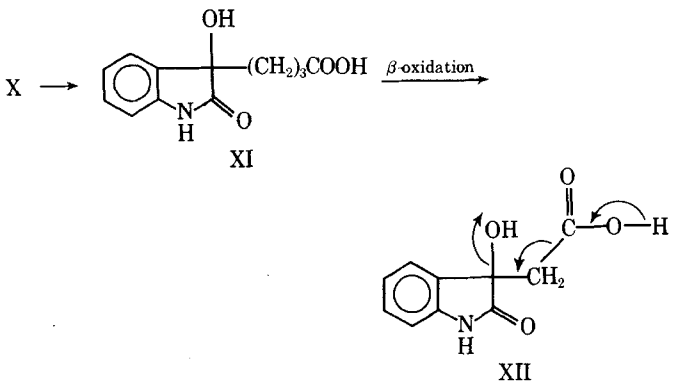
VIa: R = $COCH_3$
VIb: R = $CO_2C_2H_5$

Scheme I



IX X

Scheme II



Scheme III

(22) for the corresponding propionic lactone. However, debromination of IX failed to give X. Bioactivation of X would necessitate lactone hydrolysis followed by β-oxidation (Scheme III). 2,3-Dioxindole-3-acetic acid (XII) has been proposed as the intermediate in the enzymatic oxidation of the plant growth hormone 3-indoleacetic acid to I (23).

Compounds of Type C include XIV–XVI (Scheme IV). Treatment of oxindole with diethyl carbonate in the presence of sodium ethoxide provided 3-ethoxycarbonyloxindole (XIII). The latter readily added formaldehyde and gave the adduct XIV. Tosylation of XIV afforded the corresponding tosyl derivative XV. Compound XVI was synthesized in good yield from XIII and *N*-chloromethylphthalimide (Scheme IV).

Biological Activity—Compounds VI*a*, VI*b*, VII–IX, and XIV–XVI[1] were screened according to standard protocols[2] (24). Compounds VI*b* and VIII were evaluated against L-1210 lymphoid leukemia and were inactive at doses of 100, 200, and 400 mg/kg. The remaining six compounds were tested against P-388 lymphocytic leukemia and were inactive at 50, 100, and 200 mg/kg.

One interpretation of the data is that I is devoid of antitumor activity. Other possible explanations include: (*a*) unfavorable distribution of the latentiated forms chosen, (*b*) inappropriate biological test systems, and (*c*) bioactivation before reaching the tumor site. Further investigation is required to determine which, if any, of these explanations is correct.

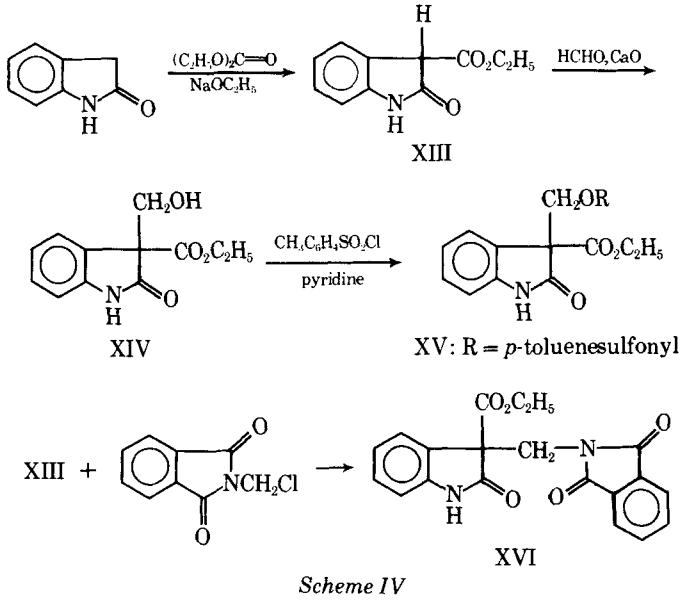
EXPERIMENTAL[3]

3-Bromo-3-oxindoleacetic Acid (VIII)—This compound was prepared according to Hinman and Bauman (21) and recrystallized from acetonitrile, mp 153.5–155.5° [lit. (21) mp 152–153°].

3-Methylaminomethyleneoxindole (IV)—A mixture of 1.0 g (4.34 mmoles) of 1-acetyl-3-ethoxymethyleneoxindole (18) in 20 ml of 30% aqueous methylamine was stirred at ambient temperature for 2.5 hr (Scheme I). The resulting precipitate was filtered and dried and amounted to 690 mg of solid, mp 230–232° [lit. (19) mp 235°]. Its IR and UV spectra agreed with the reported (19) spectra.

3-[(*N*-Methylacetamido)methyl]oxindole (VI*a*)—This compound was obtained by hydrogenation of V*a* (Scheme I) according to the recorded procedure (19), mp 134–136° [lit. (19) mp 135°]; NMR ($CDCl_3$): δ 1.92, 2.09 (2 s, 3H, CH_3C=O), 3.02, 3.05 (2 s, 3H, NCH_3), 3.58–4.17 (m, 3H, CH_2 and aliphatic CH), 6.75–7.50 (m, 4H, ArH), 9.36, and 9.67 (2 broad s, 1H, NH) ppm.

3-[(*N*-Ethoxycarbonyl-*N*-methylamino)methylene]oxindole (V*b*)—To a stirred, cooled solution of 4.0 g (0.023 mole) of IV in 32 ml of dry pyridine was added dropwise 12 ml of ethyl chloroformate (Scheme I). The solution was then warmed on the steam bath for 15 min and poured onto ice. The pale-yellow solid was filtered and washed well with cold water. The dried product, 5.24 g (93%), was recrystallized from 95% ethanol, mp 201–203°; IR ($CHCl_3$): 1710 (C=O) cm^{-1}; NMR (dimethyl sulfoxide-d_6): δ 1.28 (t, 3H, CCH_3), 3.43 (s, 3H, NCH_3), 4.30 (q, 2H, OCH_2), 6.73–7.68 (m, 4H, ArH), 7.90 (s, 1H, =CHN), and 10.47 (broad s, 1H, NH) ppm.



Scheme IV

Anal.—Calc. for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.14; H, 5.84; N, 11.20.

3 - [(*N* - Ethoxycarbonyl - *N* - methylamino)methyl]oxindole (VI*b*)—A slurry of 2.5 g (0.0102 mole) of V*b* in 200 ml of 95% ethanol containing 250 mg of 10% palladium-on-carbon catalyst was hydrogenated in a Parr apparatus (Scheme I). Hydrogen uptake stopped after 30 min, and the solution was filtered and evaporated under reduced pressure to give a colorless oil in quantitative yield. The oil was dried over phosphorus pentoxide in a drying pistol at 0.1 mm Hg for 2 hr. TLC on silica gel (ethyl acetate) exhibited a single spot, R_f 0.52; IR ($CHCl_3$): 1700 (C=O) cm^{-1}; NMR ($CDCl_3$): δ 1.18, 1.23 (overlapping t, 3H, CCH_3), 3.0 (s, 3H, NCH_3), 3.50–4.35 (m, 5H, OCH_2, CCH_2, and CH), 6.78–7.44 (m, 4H, ArH), and 9.70 (broad s, 1H, NH) ppm.

Anal.—Calc. for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28. Found: C, 62.93; H, 6.54; N, 11.34.

Ethyl 2,3-Dioxindolyl-3-acetate (VII)—Compound VII was obtained *via* a Reformatsky reaction between isatin and ethyl bromoacetate by the method of Hallmann (20). The product was recrystallized from benzene and yielded colorless crystals, mp 111–111.5° [lit. (20) mp 102°]; mass spectrum: *m/e* 235 (M^+); NMR ($CDCl_3$): δ 1.13 (t, 3H, CCH_3), 2.98 (s, 2H, CH_2C=O), 4.08 (q, 2H, OCH_2), 4.82 (s, 1H, OH), 6.76–7.56 (m, 4H, ArH), and 9.02 (broad s, 1H, NH) ppm.

5-Bromo-2,3-dioxindole-3-butyric Lactone (IX)—This compound was prepared according to a previous procedure (22) (Scheme II) for the preparation of the propionic lactone analog. The pale-yellow solid obtained from filtration was air dried overnight and amounted to 2.15 g, mp 198–203°. From the ether extraction, an additional 0.33 g was obtained, mp 204–208°, giving a total of 2.48 g (79%). Recrystallization from aqueous methanol afforded white crystals, mp 208–211°; IR (KBr): 1720 and 1750 (C=O) cm^{-1}; NMR (dimethyl sulfoxide-d_6): δ 1.42–2.94 (m, 6H, $CH_2CH_2CH_2$) and 6.75–7.92 (m, 3H, ArH) ppm.

Anal.—Calc. for $C_{12}H_{10}BrNO_3$: C, 48.67; H, 3.40; N, 4.73. Found: C, 48.48; H, 3.60; N, 4.66.

Attempted Debromination of IX—A mixture of 1.0 g (3.37 mmoles) of IX, 350 mg of 5% palladium-on-charcoal catalyst, 4 ml of dry triethylamine, and 75 ml of absolute ethanol was hydrogenated in a Parr apparatus for 2 hr (Scheme II). Workup gave 100 mg of crystals, mp 160.5–163.5°. A second crop of 65 mg, mp 164.5–168°, was also obtained. Spectroscopic evidence indicated that this compound was a carboxylic acid and it was not investigated further.

3-Ethoxycarbonyloxindole (XIII)—To a hot solution containing 10.5 g (0.079 mole) of oxindole, 40 ml of diethyl carbonate (~0.32 mole), and 50 ml of dry toluene was added dropwise an ethanolic solution of sodium ethoxide [from 1.97 g (0.085 g-atom) of sodium in 30 ml of absolute ethanol] (Scheme IV). The flask contained a mechanical stirrer and was equipped for distillation. Moisture was excluded by means of a drying tube. The rate of addition was controlled to allow a smooth rate of distillation of the ethanol–toluene azeotrope (bp 76.7°).

Near the end of the addition, a heavy precipitate appeared and an additional 75 ml of toluene was added. Heating was continued until the boiling point of the distillate fell below 76°. The resulting solid was fil-

[1] NSC 246030–246037, respectively.

[2] National Cancer Institute.

[3] Melting points were determined with a Fisher-Johns melting-point apparatus and are uncorrected. The structures of the products were confirmed by their IR and NMR spectra. IR spectra were obtained on a Beckman IR-8 spectrophotometer. NMR spectra were determined on a Varian A-60A spectrometer, using tetramethylsilane as the internal reference. Microanalysis were performed by Dr. Kurt Eder, Geneva, Switzerland.

tered, washed with toluene, slurried in water, and acidified in the cold to pH 3–4. The mixture was extracted three times with 100-ml portions of ether, and the combined extract was dried over magnesium sulfate. Concentration of the solution gave 11.5 g (71%) of an oil that slowly solidified.

Trituration with ligroin and recrystallization from cyclohexane–benzene gave crystals, mp 91–93°. A second recrystallization from the same solvent system afforded the analytically pure product, mp 93–94°; IR ($CHCl_3$): 1725 (C=O) cm^{-1}; NMR ($CDCl_3$ containing D_2O and Na_2CO_3): δ 1.27 (t, 3H, CH_3), 4.25 (q, 2H, OCH_2), and 6.75–7.60 (m, 4H, ArH) ppm. Both the NH and 3-H exchange under these conditions.

Anal.—Calc. for $C_{11}H_{11}NO_3$: C, 64.38; H, 5.40; N, 6.83. Found: C, 64.50; H, 5.44; N, 6.87.

3-Ethoxycarbonyl-3-hydroxymethyloxindole (XIV)—To a cooled solution containing 5.7 g (0.0278 mole) of 3-ethoxycarbonyloxindole and 100 mg of calcium oxide in 150 ml of 70% aqueous ethanol was added dropwise a mixture of 2.26 ml of 40% aqueous formaldehyde in 5 ml of water (Scheme IV). The resulting solution was kept at room temperature overnight. The mixture was acidified with 6.5 ml of 5% aqueous hydrochloric acid, followed by addition of 50 ml of 95% ethanol. The solution was filtered and evaporated under reduced pressure at 35° to give a solid. Recrystallization from acetonitrile afforded 2.5 g (38%) of crystals, mp 149–152° dec. (with evolution of formaldehyde); IR (KBr): 1720 (C=O) cm^{-1}; mass spectrum: *m/e* 235 (M^+); NMR (dimethyl sulfoxide-d_6): δ 1.08 (t, 3H, CH_3), 3.74 (m, 4H, 2 CH_2), 5.05 (t, 1H, OH), and 6.85–7.53 (m, 4H, ArH) ppm.

Anal.—Calc. for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.95. Found: C, 61.15; H, 5.65; N, 6.00.

3 - Ethoxycarbonyl - 3 - *p* - toluenesulfonyloxymethyloxindole (XV)—A mixture of 2.11 g (0.00897 mole) of XIV and 3.43 g (0.018 mole) of *p*-toluenesulfonyl chloride in 75 ml of pyridine was stored at 0° for 46 hr. The rose-colored solution was poured onto 100 g of ice and stirred; a pink solid was collected by filtration. After drying *in vacuo*, 1.65 g (47%) of the tosylate was obtained, mp 155–157°. Recrystallization from 95% ethanol gave colorless crystals, mp 163–164.5°; IR ($CHCl_3$): 1730 (C=O) cm^{-1}; NMR ($CDCl_3$): δ 1.14 (t, 3H, $OCCH_3$), 2.42 (s, 3H, $ArCH_3$), 4.18 (q, 2H, O=$COCH_2$), 4.58 (d, 1H, J = 10 Hz, SO_2OCH_2), 4.83 (d, 1H, J = 10 Hz, SO_2OCH_2), 6.78–7.85 (m, 8H, ArH), and 8.93 (broad s, 1H, NH) ppm.

Anal.—Calc. for $C_{19}H_{19}NO_6S$: C, 58.61; H, 4.92; N, 3.60. Found: C, 58.74; H, 4.99; N, 3.61.

3-Ethoxycarbonyl-3-phthalimidomethyloxindole (XVI)—To a slurry of 368 mg (7.68 mmoles) of a 50% sodium hydride dispersion in mineral oil in 40 ml of dimethylformamide was added 1.28 g (6.25 mmoles) of XII portionwise at room temperature (Scheme IV). After stirring for 15 min, 1.22 g (6.26 mmoles) of *N*-chloromethylphthalimide (25) was added. The dark-red color of the mixture was slowly discharged upon stirring at room temperature overnight. Then the solution was poured onto 100 g of ice, and the precipitate was filtered. After drying, 1.95 g (85%) of solid was obtained, which was recrystallized from benzene–carbon tetrachloride, mp 202–203°; IR ($CHCl_3$): 1720 and 1770 (C=O) cm^{-1}; mass spectrum: *m/e* 364 (M^+); NMR ($CDCl_3$): δ 1.19 (t, 3H, CH_3), 4.25 (q, 2H, OCH_2), 4.50 (d, 1H, J = 14.5 Hz, NCH_2), 4.83 (d, 1H, J = 14.5 Hz, NCH_2), 6.75–7.93 (m, 8H, ArH), and 8.72 (broad s, 1H, NH) ppm.

Anal.—Calc. for $C_{20}H_{16}N_2O_5$: C, 65.93; H, 4.43; N, 7.69. Found: C, 65.95; H, 4.39; N, 7.82.

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