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ACKNOWLEDGMENTS

Supported by Grant E-792 from the Robert A. Welch Foundation.

NOTES

Synthesis and Antibacterial and Anticancer Evaluations of α-Methylene-γ-butyrolactones

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Received April 7, 1980, from the *Center for Health Sciences, Lehigh University, Bethlehem, PA 18015.* Accepted for publication July 17, 1980.

Abstract □ Nine new α-methylene-γ-butyrolactones were synthesized by the Reformatsky condensation of ethyl α-bromomethylacrylate with ketones, aldehydes, and an epoxide. A unique spirobutyrolactone class was prepared by reaction of the zinc alkyl derivative and *N*-methylisatins. The compounds were evaluated against L-1210 and P-388 leukemia and the 9KB carcinoma of the nasopharynx. They also were screened in a microbiocidal and an antifungal assay. The spiro methylene lactone of 5-iodo-*N*-methylisatin displayed activity in the P-388, 9KB, and antifungal screens.

Keyphrases □ α-Methylene-γ-butyrolactones—synthesis and evaluation for antibacterial and antineoplastic activity □ Antibacterial activity, potential—evaluation of α-methylene-γ-butyrolactones □ Antineoplastic activity, potential—evaluation of α-methylene-γ-butyrolactones

Recognized as the active moiety in a wide variety of antineoplastic sesquiterpene lactones (1, 2), the α-methylene-γ-butyrolactone structure has been synthetically incorporated in numerous drug candidates in the search for anticancer activity (3, 4). Many synthetic approaches have been used (5), and new methods are reported regularly (6, 7) since many of the model substances are active.

The application of the Reformatsky method by Öhler *et al.* (8) is a facile, one-step conversion of aldehydes and ketones to the requisite lactones with the greatest potential of altering the functionality at C-5 (Scheme I). This condensation of the zinc alkyl derivative of ethyl α-bromomethylacrylate with *N*-methylisatins (II*a* and II*b*) also yields a unique class of spirolactones (V*a* and V*b*) (Scheme II). Nine lactones were synthesized in 25–59% yields using this procedure (Table I).

EXPERIMENTAL[1]

***p*-(Pyrrolidinosulfonamido)benzaldehyde (I*d*)**—Compound I*d* was obtained by the dropwise addition of 2.78 g (39.2 mmoles) of anhydrous pyrrolidine in 10 ml of acetone to 6.00 g (19.5 mmoles) of *p*-chlorosulfonylbenzaldehyde diacetate (9) in 25 ml of acetone. The medium was stirred for 1 hr, the acetone was removed *in vacuo*, and the residue was dissolved in methylene chloride and washed with 20 ml of 10% aqueous HCl. The solvent was removed by distillation, and the crystalline diacetate was hydrolyzed by refluxing for 1 hr in a solution of 25 ml of ethanol, 25 ml of water, and 3 ml of concentrated sulfuric acid. The solution was chilled and it deposited the aldehyde as white crystals, which were filtered, recrystallized twice from ethanol, and dried *in vacuo* to yield 3.35 g (50%) of I*d*, mp 109–111°; IR (mineral oil): 1710 (C=O) cm^{-1}; NMR (deuterochloroform): δ 1.60–1.95 (m, 4H, CH_2CH_2), 3.16–3.50 (m, 4H, CH_2NCH_2), 8.1 (broad s, 4H, aromatic H), and 10.17 (s, 1H, CHO) ppm.

Anal.—Calc. for $C_{11}H_{13}NOS_2$: C, 55.19; H, 5.47; N, 5.85. Found: C, 55.16; H, 5.68; N, 5.71.

***p*-(Pyrrolidinosulfonamido)acetophenone (I*f*)**—Compound I*f* was prepared by the addition of 3.50 g (49.3 mmoles) of anhydrous pyrrolidine in 10 ml of acetone to 5.00 g (22.9 mmoles) of *p*-chlorosulfonylacetophenone (10) in 25 ml of acetone. After 2 hr of refluxing and stirring, the solution was poured into 75 ml of cold water. The resulting precipitate was collected, washed with cold water, dried *in vacuo*, and recrystallized twice from ethanol to yield 4.30 g (74%) of I*f* as pale-yellow needles, mp 142–144°; IR (mineral oil): 1690 (C=O) cm^{-1}; NMR (deuterochloroform): δ 1.65–2.00 (m, 4H, CH_2CH_2), 2.70 (s, 3H, $COCH_3$), 3.20–3.50 (m, 4H, CH_2NCH_2), and 7.85–8.30 (m, 4H, aromatic H) ppm.

Anal.—Calc. for $C_{12}H_{15}NO_3S$: C, 56.95; H, 5.97; N, 5.53. Found: C, 57.13; H, 6.16; N, 5.67.

[1] Analyses were performed by Dr. G. I. Robertson, Florham Park, N.J. Melting points were determined between glass disks on a Fisher-Johns apparatus and are uncorrected. NMR spectra were obtained on a Perkin-Elmer Hitachi R20A spectrometer and were calibrated against tetramethylsilane. IR spectra were obtained on a Perkin-Elmer 257 spectrophotometer as petroleum oil mulls.

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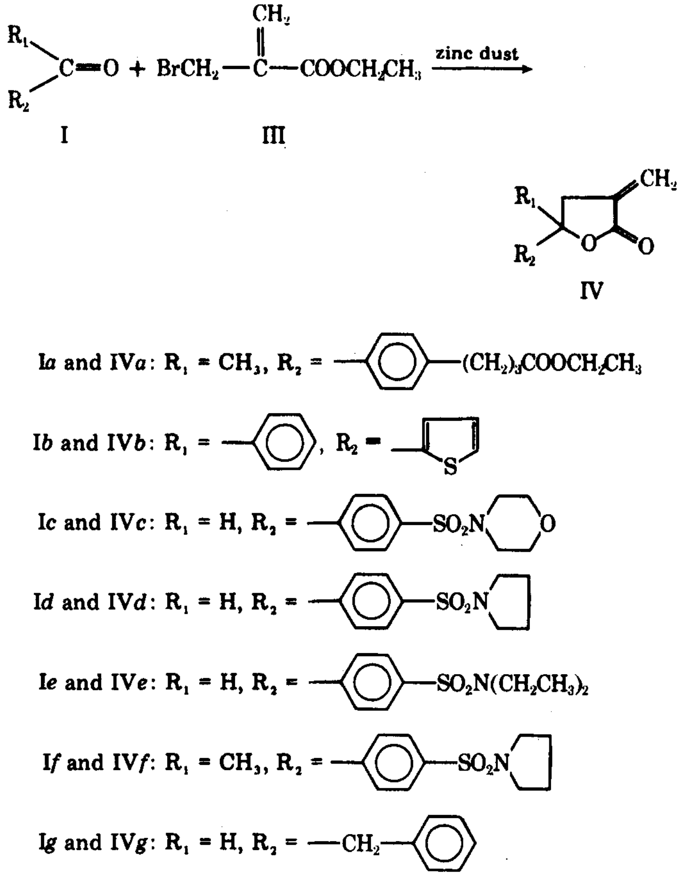
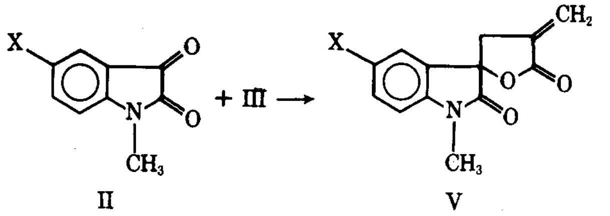


Scheme I



IIa and Va: X = H
IIb and Vb: X = I
Scheme II

Ethyl *p*-acetyl-4-phenylbutyrate (I*a*) (11), *p*-(*N,N*-diethylsulfonamido)benzaldehyde (I*e*) (9), *p*-(morpholinosulfonamido)benzaldehyde (I*c*) (9), *N*-methylisatin (II*a*) (12), and 5-iodo-*N*-methylisatin (II*b*) (12) were prepared by published methods. Phenyl 2-thienyl ketone (I*b*), phenylacetaldehyde (I*g*), and styrene oxide were obtained commercially and were used without further purification.

General Procedure for Synthesis of α-Methylene-γ-butyrolactones (IV*a*–IV*g*, V*a*, and V*b*)—A four-necked flask was fitted with a condenser with its outlet connected to a mercury bubbler, a thermometer, a dropping funnel, and a nitrogen inlet tube. The system was flame dried and charged with 25 mmoles of the requisite carbonyl compound (I*a*–I*h*, II*a*, and II*b*) or styrene oxide. To this mixture were added 25 mmoles of dry zinc dust, three crystals of iodine, and sufficient tetrahydrofuran (~10–30 ml) to effect solution of the organic component. With vigorous stirring and a constant nitrogen gas blanket, 25 mmoles of ethyl α-bromomethylacrylate (13) in 5 ml of tetrahydrofuran was added dropwise. There was an initial exothermic reaction that soon subsided.

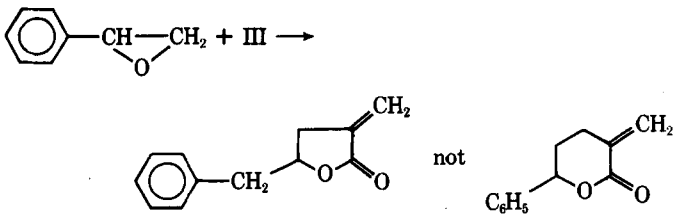
When the addition was completed, the contents of the flask were heated at reflux for 3 hr with constant stirring, cooled, and poured into 50 ml of 10% aqueous HCl. This aqueous medium was agitated for 15 min to ensure complete hydrolysis and extracted with two 30-ml portions of methylene chloride. The extracts were washed once with water, dried over magnesium sulfate, and evaporated *in vacuo* to give the title compounds, which solidified (except IV*a* and IV*g*) from the initially oily phase. The liquids (IV*a* and IV*g*) were purified by vacuum distillation, after which IV*g* solidified as white needles. All solids were recrystallized from ethanol-benzene (1:1) to analytical purity; the yields, physical properties, and analytical results are given in Table I.

All products were submitted for anticancer screening in the National Cancer Institute L-1210, P-388, and 9KB assays (Table I) and for evaluation against a Gram-positive (*Staphylococcus aureus*, ATCC 6538), a Gram-negative (*Escherichia coli*, ATCC 8739), and a fungal (*Candida*

Table I—Physical and Pharmacological Properties of Methylene Lactones

Compound	Melting Point (Boiling Point/mm Hg)	Yield, %	Formula	Analysis, % Calc.	Analysis, % Found	P-388 Leukemia[a] Dose, mg/kg	Survival	T − C Wt., g	T/C %	KB Screen[a] ED_{50}, μg/ml
IV*a*	(225–227°/1.9)	33	$C_{18}H_{22}O_4$	C 71.50	71.28	—	—	—	—	21.0
				H 7.34	7.44					
IV*b*	108–110°	25	$C_{15}H_{12}O_2S$	C 70.29	70.18	—	—	—	—	—
				H 4.72	4.96					
IV*c*	150–151°	40	$C_{15}H_{17}NO_5S$	C 55.72	55.91	100	0/6	—	T	2.7
				H 5.30	5.50	50	4/6	−3.7	T	
				N 4.33	4.37	25	6/6	−0.8	144	
						12.5	6/6	+0.7	147	
IV*d*	112–115°	59	$C_{15}H_{17}NO_4S$	C 58.62	58.64	—	—	—	—	—
				H 5.58	5.67					
				N 4.56	4.52					
IV*e*	95–98°	52	$C_{15}H_{19}NO_4S$	C 58.22	57.98	—	—	—	—	15.0
				H 6.19	6.30					
				N 4.52	4.62					
IV*f*	142–145°	55	$C_{16}H_{19}NO_4S$	C 59.80	59.66	—	—	—	—	21.0
				H 5.96	6.15					
				N 4.36	4.29					
IV*g*	63–64°	26	$C_{12}H_{12}O_2$	C 76.57	76.44	—	—	—	—	27.0
	(131–136°/0.6)			H 6.41	6.58					
V*a*	135–137°	25	$C_{13}H_{11}NO_3$	C 68.11	67.85	—	—	—	—	—
				H 4.83	5.15					
				N 6.10	6.05					
V*b*	157–159°	34	$C_{13}H_{10}INO_3$	C 43.95	44.20	100	2/6	−4.0	T	0.28
				H 2.83	3.03	50	3/6	−9.3	T	
				N 3.94	3.84	25	5/6	−2.6	139	
						12.5	6/6	−2.4	129	

[a] All test results were provided by the National Cancer Institute. Procedures, protocols, and data interpretation were published in R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. G. Abbott, *Cancer Chemother. Rep.*, *Part 3*, 3, 7 (1972), and in "Instruction Booklet 14," Drug Evaluation Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md., 1978. The symbol T means the substance was too toxic for calculation of T/C % values.



IV*g*

Scheme III

albicans, Strain 582, Rutgers University Collection) organism according to the previously published method (14).

RESULTS AND DISCUSSION

The reaction of the Reformatsky reagent from III and styrene oxide did not yield a δ-phenyl-α-methylene-δ-valerolactone as expected from ring opening of the oxide but instead gave a 26% yield of γ-benzyl-α-methylene-γ-butyrolactone (IV*g*) (Scheme III). The mass spectrum showed intense P − 91 and 91 amu peaks corresponding to tropilium ions derived from a benzyl–carbon scission (15). Furthermore, comparison of the spectra, melting points, and mixed melting points showed that the product was identical to that obtained from phenylacetaldehyde and the zinc alkyl derivative of III. The literature contains other examples of epoxide to carbonyl rearrangements induced during Reformatsky reactions. Thus, cyclopentene oxide and cyclopentanone both gave ethyl 1-hydroxycyclopentyl acetate with ethyl α-bromozinc acetate (16). Furthermore, α-pinene oxide under Reformatsky conditions gave the same hydroxy ester as that derived from the carbonyl compound generated by prior rearrangement of the oxide (17).

PMR spectroscopy was useful in identifying the lactone products (IV*a*–IV*g*, V*a*, and V*b*). For all of the exocyclic methylene groups, two apparent triplets (J = 3 Hz) were found at δ 6.28 ± 0.1 for the proton *anti* to the carbonyl group and at δ 5.72 ± 0.08 ppm for the proton *cis* to the carbonyl group. The C-4 methylene signals were complex multiplets at 2.6–3.8 ppm. Characteristic IR bands were found at 1660 ± 5 (C=C) and 1760 ± 5 (C=O) cm^{-1} for each lactone prepared.

As was noted in other model α-methylene-γ-butyrolactones, no active analogs were found in the L-1210 lymphoid leukemia screen (3). In addition, all of the compounds, except IV*b*, were highly toxic to the test animals and could not be evaluated accurately at doses in excess of 100 mg/kg. However, in the P-388 lymphocytic leukemia screen (ascitic fluid, intraperitoneal administration to CDF_1 mice), two of the analogs (IV*c* and V*b*) were evaluated, and both met the minimal activity standards of T/C > 125%. Although toxic at 50 mg/kg, IV*c* and V*b* increased the lifespan of treated animals at lower doses by 47% (T/C = 147%) and 39%, respectively. Furthermore, in the *in vitro* assay against cells derived from human carcinoma of the nasopharynx (KB) carried in cell culture, V*b* displayed potency (ED_{50} = 0.28 μg/ml) in the approximate range of that observed for the sesquiterpene lactones from which these methylene lactones are modeled.

Compounds IV*b*–IV*e*, IV*g*, V*a*, and V*b* were evaluated in an antibacterial/antifungal assay in which zones of inhibition were measured from the edge of an assay disk impregnated with 4 mg of the test compound. At these concentrations, sulfamerazine gave a 17-mm zone against *E. coli* and IV*d* gave an 8-mm zone, but no other analogs tested appeared to be active. With *S. aureus*, IV*g* and V*a* both displayed 12-mm zones, while the standard sulfamerazine demonstrated a 4-mm region of inhibition. No other test compounds showed significant activity. Against *C. albicans*, for which sulfamerazine possessed negligible activity (~1-mm zone), IV*g* displayed an 18-mm zone of inhibition, and both IV*d* and V*b* demonstrated ~4-mm zones. All other analogs were inactive.

As Rosowsky *et al.* (18) found in a series of model methylene lactones, no particular correlation of structure and activity is evident from the data. The activity profile in the spiro indole methylene lactone (V*b*) should encourage further research in this class.

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ACKNOWLEDGMENTS

Supported in part by Grant CA-22579 from the National Cancer Institute.