TABLE I

ALKYLFURYL- AND ALKYLTHIENYLCARBINOLS

Carbinol	B.P.°, mm.	n_D	Empirical Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
Methyl(5-methyl-2-furyl)	88/18	—	$C_7H_{10}O_2$	66.6	66.5	8.0	8.1
Ethyl(5-methyl-2-furyl)	98/21	—	$C_8H_{12}O_2$	68.5	68.6	8.6	8.9
Butyl(5-methyl-2-furyl)	137/15	1.4842/27°	$C_{10}H_{16}O_2$	71.4	71.3	9.6	9.5
Isobutyl(5-methyl-2-furyl)	129/15	1.4814/27°	$C_{10}H_{16}O_2$	71.4	71.2	9.6	9.7
Amyl(5-methyl-2-furyl)	139/15	1.4701/27°	$C_{11}H_{18}O_2$	72.5	72.5	10.0	10.1
Isoamyl(5-methyl-2-furyl)	133/15	1.4739/27°	$C_{11}H_{18}O_2$	72.5	72.3	10.0	10.3
Hexyl(5-methyl-2-furyl)	151/15	1.4707/27°	$C_{12}H_{20}O_2$	73.4	73.3	10.3	10.3
β-Ethylbutyl(5-methyl-2-furyl)	145/15	—	$C_{12}H_{20}O_2$	73.4	73.2	10.3	10.5
Heptyl(5-methyl-2-furyl)	154/15	1.4808/21°	$C_{13}H_{22}O_2$	74.2	74.0	10.5	10.6
Octyl(5-methyl-2-furyl)	157/15	1.4779/26°	$C_{14}H_{24}O_2$	75.0	74.7	10.8	10.7
Butyl-2-thienyl	141/15	1.5522/27°	$C_9H_{14}OS$	63.5	63.2	8.3	8.2
Isobutyl-2-thienyl	134/15	1.5209/27°	$C_9H_{14}OS$	63.5	63.4	8.3	8.4
Amyl-2-thienyl	144/15	1.5232/27°	$C_{10}H_{16}OS$	65.2	65.0	8.8	9.0
Isoamyl-2-thienyl	138/15	1.5437/27°	$C_{10}H_{16}OS$	65.2	65.2	8.8	9.0
Hexyl-2-thienyl	160/15	1.5239/27°	$C_{11}H_{18}OS$	66.6	66.5	9.2	9.5
β-Ethylbutyl-2-thienyl	149/15	—	$C_{11}H_{18}OS$	66.6	66.5	9.2	9.3
Heptyl-2-thienyl	166/15	1.5207/21°	$C_{12}H_{20}OS$	67.9	67.8	9.5	9.5
Octyl-2-thienyl	169/15	1.5103/26°	$C_{13}H_{22}OS$	69.0	68.8	9.8	9.7
Butyl(5-methyl-2-thienyl)	145/15	1.5232/27°	$C_{10}H_{16}OS$	65.2	65.0	8.8	8.9
Isobutyl(5-methyl-2-thienyl)	137/15	1.5474/27°	$C_{10}H_{16}OS$	65.2	65.1	8.8	8.8
Amyl(5-methyl-2-thienyl)	147/15	1.5340/27°	$C_{11}H_{18}OS$	66.6	66.4	9.2	9.5
Isoamyl(5-methyl-2-thienyl)	144/15	1.5342/27°	$C_{11}H_{18}OS$	66.6	66.5	9.2	9.3
Hexyl(5-methyl-2-thienyl)	164/15	1.5310/27°	$C_{12}H_{20}OS$	67.9	67.6	9.5	9.8
β-Ethylbutyl(5-methyl-2-thienyl)	153/15	1.5362/21°	$C_{12}H_{20}OS$	67.9	67.8	9.5	9.5
Heptyl(5-methyl-2-thienyl)	171/15	1.5015/20°	$C_{13}H_{22}OS$	69.0	68.8	9.8	9.8
Octyl(5-methyl-2-thienyl)	175/15	1.5129/26°	$C_{14}H_{24}OS$	70.0	69.7	10.1	10.3

TABLE II

ALKYLARYLCARBINOLS

Carbinol	B.P., mm.	n_D	Empirical Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
Amyl-*p*-tolyl	150/15	1.5065/27°	$C_{13}H_{20}O$	81.2	81.0	10.5	10.6
Isoamyl-*p*-tolyl	147/15	1.5063/27°	$C_{13}H_{20}O$	81.2	81.3	10.5	10.8
Hexyl-*p*-tolyl	169/15	1.5092/27°	$C_{14}H_{22}O$	81.5	81.2	10.8	10.9
β-Ethylbutyl-*p*-tolyl	158/15	1.5128/21°	$C_{14}H_{22}O$	81.5	81.6	10.8	10.7
Heptyl-*p*-tolyl	177/15	1.5008/20°	$C_{15}H_{24}O$	81.8	81.6	11.0	11.3
Octyl-*p*-tolyl	182/15	1.4965/27°	$C_{16}H_{26}O$	82.0	81.9	11.2	11.3
Methyl-3,4-xylyl	150/18	—	$C_{10}H_{14}O$	80.0	79.8	9.4	9.7
Ethyl-3,4-xylyl	140/18	—	$C_{11}H_{16}O$	80.4	80.3	9.8	9.9
Methyl-2,5-xylyl	127/16	—	$C_{10}H_{14}O$	80.0	79.8	9.4	9.5
Ethyl-2,5-xylyl	134/18	—	$C_{11}H_{16}O$	80.4	80.3	9.8	9.9

Tolualdehyde and 3,4- and 2,5-dimethylbenzaldehyde were prepared from *p*-methyl-, 3,4-dimethyl- and 2,5-dimethylbenzyl chloride with hexamethylenetetramine by means of the Sommelet reaction (60–65% yields).

Grignard reactions. To an ice-cooled ethereal solution of the appropriate alkylmagnesium bromide (1.15 moles), the aldehyde (1 mole, dissolved in its volume of anhydrous ether) was added in small portions with stirring. The reaction was completed by a brief heating on the water bath; after cooling, the reaction product was treated with an ice-cooled aqueous solution of ammonium chloride, the ethereal layer washed with water and dried over sodium sulfate, the solvent distilled, and the residue vacuum-fractionated.

INSTITUT DU RADIUM
26, RUE D'ULM
PARIS (VE), FRANCE

Potential Anticancer Agents. XLIX.[1] Analogs of Chlorambucil. VII.[2] Nitrogen Mustards Derived from Cinnamic Acid

W. A. SKINNER, MARC G. M. SCHELSTRAETE, AND B. R. BAKER

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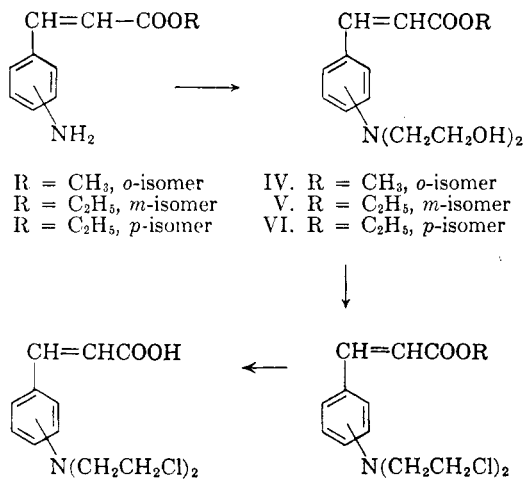
The previous paper of this series[2] described the synthesis of some ring isomers of Chlorambucil (XV) and Norchlorambucil. In the synthesis of *o*-Norchlorambucil, *o*-[bis(2-chloroethyl)amino]hy-

drocinnamic acid, the key intermediate was methyl *o*-[bis(2-hydroxyethyl)amino]cinnamate (IV). The availability of IV suggested that it be converted to *o*-cinnamic acid mustard (VII). Surprisingly, this compound showed good activity against Sarcoma 180, Adenocarcinoma 755, and Leukemia L-1210, a tumor spectrum more like phenylalanine mustard (XIII) than like Chlorambucil (XV), which has barely perceptible activity at the maximum tolerated doses against these three tumors. Due to the unusual antitumor activity of VII, the *p*- and *m*-cinnamic acid mustards (VIII and IX) were also synthesized for evaluation.



I. R = CH_3, *o*-isomer
II. R = C_2H_5, *m*-isomer
III. R = C_2H_5, *p*-isomer

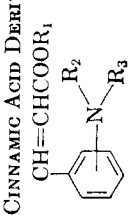
IV. R = CH_3, *o*-isomer
V. R = C_2H_5, *m*-isomer
VI. R = C_2H_5, *p*-isomer

VII. *o*-isomer
VIII. *m*-isomer
IX. *p*-isomer

X. R = CH_3, *o*-isomer
XI. R = C_2H_5, *m*-isomer
XII. R = C_2H_5, *p*-isomer

Ethyl *m*- and *p*-aminocinnamate (II and III) were readily prepared by reduction of the corresponding nitro esters with zinc and ammonium chloride, as previously described for the *o*-isomer (I).[2] Hydroxyethylation of II and III by the method used for the preparation of IV[2] gave a 63% yield of V as its crystalline hydrochloride and a 61% yield of VI as the crystalline base. Chlorination of the hydroxyethyl derivatives (IV–VI) with phosphorus oxychloride to the chloroethyl derivatives (X–XII) proceeded smoothly. However, as could be anticipated, the cinnamic esters (X–XII) were acid-hydrolyzed with more difficulty to VII–IX than the corresponding hydrocinnamic esters.[2] Thus, after chlorination it was necessary to complete the hydrolysis by heating with aqueous hydrochloric acid. The crystalline, chromatographically pure mustards (VII–IX) were obtained in

TABLE I CINNAMIC ACID DERIVATIVES



Compound No.[a]	R_1	$R_2 = R_3$	Isomer	Method[b]	Yield, %	M.P.	R_f[c]	Calcd. C	Calcd. H	Calcd. Cl	Calcd. N	Found C	Found H	Found Cl	Found N
II	C_2H_5	H	*m*	A	65	63–64[d]	0.41								
III	C_2H_5	H	*p*	A	77	68–69[e]									
IV[f]	C_2H_5	$—CH_2CH_2OH$	*m*	B	63	89–126 dec.	0.76[g]	57.0	7.03	11.2	4.44	57.0	7.24	11.3	4.35
V	C_2H_5	$—CH_2CH_2OH$	*p*	B	61	75–76[h]	0.71	64.5	7.58		5.01	64.6	7.58		5.15
VII	H	$—CH_2CH_2Cl$	*o*	C	55	123–125[i]	0.44	54.2	5.24	24.6	4.87	54.5	5.49	24.4	4.84
VIII	H	$—CH_2CH_2Cl$	*m*	C	61	175–178[j]	0.68	54.2	5.24	24.6	4.87	54.4	5.30	24.3	4.91
IX	H	$—CH_2CH_2Cl$	*p*	C[k]	56	189–191[j]	0.62[l]	54.2	5.24	24.6	4.87	54.3	5.31	24.5	4.82

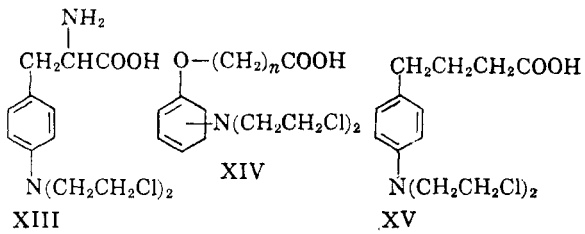
[a] Infrared spectra were compatible with the assigned structures. [b] Method A is the reduction of the corresponding nitrocinnamate with zinc and ammonium chloride as described for methyl *o*-aminocinnamate.[2] Method B is hydroxyethylation of the corresponding amine as described for methyl *o*-[bis(2-hydroxyethyl)amino]cinnamate.[2] For Method C, see Experimental. [c] Paper chromatograms were run by the descending technique on Schleicher and Schuell No. 2495 acetylated paper with benzene–methanol–water (2:6:1). Spots were detected by examination under ultraviolet light. [d] Lit.[5] m.p. 63–64°. [e] Lit.[6] m.p. 68–69°. [f] Hydrochloride. [g] Both the free base and hydrochloride had the same R_f, indicating the ease of dissociation of the salt to the base. [h] Recrystallized from ether. [i] Recrystallized from petroleum ether (b.p. 30–60°). [j] Recrystallized from toluene–petroleum ether (b.p. 30–60°). [k] Modified by using 2:1 benzene–phosphorus oxychloride at the b.p. for chlorination. [l] The corresponding ethyl ester (XII) had R_f 0.28.

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center.

(2) For paper VI on analogs of Chlorambucil see W. A. Skinner, M. G. M. Schelstraete, and B. R. Baker, *J. Org. Chem.*, **26**, 1554 (1961), Paper XLVIII of this series.

55–61% over-all yield from the bis(hydroxyethyl)-amino esters (IV–VI).

The *m*-mustard of cinamic acid (VIII) was also active against Sarcoma 180, Adenocarcinoma 755, and Leukemia L-1210; testing on the *p*-mustard (IX) is as yet incomplete.[3] It is of considerable theoretical interest that VII and VIII differ in tumor spectrum from Chloroambucil (XV) but are similar to phenylalanine mustard (XIII). In addition, mustards derived from phenoxyacetic acid and phenoxypropionic acid (XIV)[4] are more like phenylalanine mustard (XIII) in their tumor



spectra than like Chlorambucil (XV).[3] The cinnamic acid mustards (VII–IX), phenylalanine mustard (XIII), and the phenoxyalkanoic acid mustards (XIV) have in common a side chain functional group in addition to the usual carboxyl of Chlorambucil (XV); therefore, synthesis of other analogs of Chlorambucil (XV), and perhaps phenylalanine mustard (XIII), with other functional groups in the side chain for test evaluation would be warranted, since it would appear that the extra functional group causes a change in metabolism or tissue absorption of the candidate drug.

EXPERIMENTAL[7]

o-[*Bis*(*2-chloroethylamino*)]*cinnamic acid* (VII). *Method C.* Methyl *o*-[bis(2-hydroxyethyl)amino]cinnamate (IV)[2] was treated with phosphorus oxychloride as previously described for the preparation of *m*-[bis(2-chloroethyl)amino]hydrocinnamic acid,[2] except that the aqueous solution of decomposed phosphorus oxychloride was worked up by extraction without standing for 20 hr., since conversion of X to VII was slow under these conditions. The crude ester (X) was then refluxed with 12*N* hydrochloric acid for 15 min. and worked up as described for mustards of phenoxyacetic acid.[4] Evaporation of the benzene extract *in vacuo* gave a 55% yield of VII, m.p. 123–125°; $\lambda_{max(\mu)}^{Nujol}$ 3.50 (broad acidic OH); 5.90 (carboxyl C=O); 6.15, 6.25, 6.70 (C=C, aryl), 13.1 (*ortho*-disubstituted benzene). An analytical sample, m.p. 123–125°, was prepared by recrystallization from petroleum ether (b.p. 30–60°). See Table I for analytical data.

(3) The assays were performed at this Institute by Dr. J. Greenberg and staff under contract to the Cancer Chemotherapy National Service Center.

(4) W. A. Skinner, A. P. Martinez, and B. R. Baker, *J. Org. Chem.*, **26**, 152 (1961), Paper XLVI of this series.

(5) H. Salkowski, *Ber.*, **28**, 1917 (1895).

(6) F. Mayer, H. Philips, F. W. Ruppert, and A. T. Schmitt, *Ber.*, **61**, 1966 (1928).

(7) Melting points were taken on a Fisher-Johns block and are uncorrected.

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DEPARTMENT OF BIOLOGICAL SCIENCES
STANFORD RESEARCH INSTITUTE
MENLO PARK, CALIF.

Stereospecific Microbiological Reduction of 3-Keto-1,4-pregnadienes to 3-Keto-1-pregnenes

GEORGE GREENSPAN,[1] CARL P. SCHAFFNER, WILLIAM CHARNEY, MARGARET JEVNIK GENTLES, AND HERSHEL L. HERZOG

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At the dawn of the application of microorganisms to the transformation of steroidal substrates, Mamoli and his collaborators[2] observed that testosterone and 4-androstene-3,17-dione were transformed by "putrefactive bacteria" into 4,5α-dihydro derivatives and by *B. putrificus* into 4,5β-dihydro derivatives. The application of these earlier discoveries in recent times has been hampered by the lack of availability and incomplete identification of the organisms involved.

Other workers[3a,b] have since demonstrated the reduction of Δ^4-3-ketosteroids to 4,5α- and 4,5β-dihydroanalogs, incidental to hydroxylation elsewhere in the substrates employed.

We have now found that *Streptomyces sp.* W 3808 (Waksman Collection, Institute of Microbiology, Rutgers University) transforms selected 3-keto-1,4-pregnadienes into 3-keto-1-pregnenes. Incubation of prednisone for 112 hours with a 112-hour growth culture afforded, after chloroform extraction and silicic acid chromatography, a less polar product (I), $\lambda_{max}^{CH_3OH}$ 225 mμ (ε 6600). The infrared spectrum of I was essentially that of 1-pregnene-17α,21-diol-3,11,20-trione.[4] To complete the identification, I was acetylated with acetic anhydride in pyridine solution to yield a 21-acetate, whose infrared spectrum matched that of an authentic sample.[5]

(1) Present address, Wyeth Institute for Medical Research, Radnor, Pa.

(2) For the pertinent references see F. Fischer, *Newer Methods of Preparative Organic Chemistry*, Interscience, New York, 1948, p. 184–190.

(3a) D. Perlman, E. Titus, and J. Fried, *J. Am. Chem. Soc.*, **74**, 2126 (1952). (b) D. H. Peterson, H. C. Murray *et al.*, *J. Am. Chem. Soc.*, **74**, 5933 (1952); **75**, 412 (1953); **76**, 3174 (1954).

(4) Prepared by hydrolysis of authentic 21-acetate of I (Ref. 5).

(5) V. R. Mattox and E. C. Kendall, *J. Biol. Chem.*, **188**, 287 (1951).