Experimental Section[a]

2-Amino-4-*n*-butylamino-5-(*p*-chlorophenylazo)pyrimidine-6-isothiouronium Chloride (II).—A mixture of 10.2 g (0.03 mole) of I, 2.44 g (0.03 mole) of thiourea and 150 ml of *t*-butyl alcohol was refluxed with stirring for 1 hr. After cooling to room temperature, 100 ml of acetone was added to the mixture which was then cooled to 5°. The separated, yellow needles were washed with acetone and dried to give 9.75 g (78.2%) of the analytically pure product; mp 203–204°; λ_{max}^{EtOH} 225 mμ (ε 29,500), 261 (17,400), 306 (38,100).

Anal. Calcd for $C_{15}H_{20}Cl_2N_8S$: C, 43.37; H, 4.86; Cl, 17.07; N, 26.98; S, 7.72. Found: C, 43.61; H, 4.90; Cl, 16.95; N, 27.14; S, 7.81.

2-Amino-4-*n*-butylamino-5-(*p*-chlorophenylazo)-6-methoxypyrimidine (III).—A freshly prepared solution of sodium methoxide, from 0.81 g (0.035 mole) of sodium and 60 ml of dry methanol, was added with stirring to a suspension of 10.2 g (0.03 mole) of I in 250 ml of dry methanol, and the reaction mixture was refluxed under a $CaCl_2$ tube for 3 hr. Water (1 ml) was added, and the methanol was distilled until crystallization began; the mixture was cooled gradually to 0°. The separated, yellow needles were washed with cold methanol and dried to give 9.9 g (98%) of the desired product, mp 117–118°. A sample was recrystallized from methanol: mp 118°; λ_{max}^{EtOH} 255 mμ (ε 13,500), 304 (5120), 388 (32,400).

Anal. Calcd for $C_{15}H_{19}ClN_6O$: C, 53.80; H, 5.73; Cl, 10.59; N, 25.10. Found: C, 53.77; H, 5.60; Cl, 10.70; N, 25.00.

2-Amino-4-*n*-butylamino-5-(*p*-chlorophenylazo)-6-pyrimidinethiol (IV). A. From I.—A suspension of 20.3 g (0.06 mole) of I, 14 g (~0.15 mole) of NaHS + aq. (Fisher) in 1700 ml of dry ethanol was refluxed under $CaCl_2$ with vigorous stirring for 12 hr. Another charge of NaHS was then added, and the reaction was continued for an additional 12 hr. The orange solid was separated from the hot reaction mixture by filtration, washed with six 100-ml portions of water followed by three 100-ml portions of ethanol and three 100-ml portions of ether, and dried to give 9.8 g (48.5%) of analytically pure product: mp 212–213°; λ_{max}^{EtOH} 226 mμ (ε 20,100), 261 (8710), 306 (24,900), 427 (18,100).

Anal. Calcd for $C_{14}H_{17}ClN_8S$: C, 49.91; H, 5.10; Cl, 10.52; N, 24.96; S, 9.51. Found: C, 50.05; H, 5.19; Cl, 10.46; N, 25.09; S, 9.53.

B. From II.—A suspension of the thiouronium salt (II) in excess 2 *N* NaOH was vigorously stirred for 15 min at room temperature until a clear solution was obtained. On neutralization with HCl, IV precipitated in quantitative yield.

2-Amino-4-*n*-butylamino-5-(*p*-chlorophenylazo)-6-pyrimidinol (IX).—To a suspension of 10.9 g (0.06 mole) of VIII,[b] 60 g of anhydrous sodium acetate, and 300 ml of 50% aqueous acetic acid at 1–3° was added dropwise over 5 min, a solution of *p*-chlorophenyldiazonium chloride (from 8.3 g (0.065 mole) of *p*-chloroaniline, 4.5 g (0.065 mole) of $NaNO_2$, 15 ml of water, and 120 ml of 2 *N* HCl). The mixture was stirred for 1 hr at 2–3°, and at room temperature for 13 hr. The precipitated, yellow product was washed with water, dried, and recrystallized from a mixture of dimethylformamide and ethanol to give 15.1 g (78%) of yellow needles, mp 273–274°, λ_{max}^{EtOH} 243 mμ (ε 12,500), 251 (12,300), 258 sh (9960), 384 (21,100).

Anal. Calcd for $C_{14}H_{17}ClN_6O$: C, 52.41; H, 5.66; N, 26.12; Cl, 11.04. Found: C, 52.61; H, 5.80; N, 26.18; Cl, 11.11.

4-*n*-Butylamino-2,5-diamino-6-chloropyrimidine (V).[3]—In a 2-l., three-necked, round-bottom flask fitted with a mechanical stirrer, reflux condenser, and two 100-ml, pressure-equalizing, addition funnels, set up in such a manner that a slow stream of nitrogen could blanket the contents of the flask and the funnel, was placed 40 g (0.61 g-atom) of zinc (which had been activated by the method of Baer and Buchner[3]), 378 ml of water, and 278 ml of ethanol. The rapidly stirred mixture was then brought to reflux under a slow stream of nitrogen, and a warm solution of 15.1 g (0.44 mole) of I in 80 ml of dimethylformamide was added dropwise at such rate that a finely divided suspension was formed. Following the addition of I, 37.8 ml of glacial acetic acid was added in a similar manner over 0.5 hr. After addition of the acid, the mixture was stirred with refluxing under nitrogen for 1 hr, then filtered rapidly while hot. The filtered residue (largely zinc) was washed with three 30-ml portions of ethanol, the washings were added to the filtrate, and the combined solution was cooled under nitrogen to 3–5° in an ice bath. After addition of 6 *N* NaOH to the cold, red solution to a pH of 10, a mixture of 1 g of decolorizing charcoal and 20 g of Celite was added (stirring). After 10 min, the cold suspension was filtered under nitrogen through a bed of Celite, the residue was washed with three 50-ml portions of ethanol, and the filtrate and washings were combined. The red solution was brought to pH 7 by the addition of glacial acetic acid, concentrated *in vacuo* to 400 ml, and cooled to 5°. The dark red, crystalline solid was filtered and immediately stirred with 150 ml of 2 *N* HCl. After the undissolved material was removed by filtration, the filtrate was brought to pH 5 and again filtered. To the light yellow solution was added 6 *N* NaOH with stirring until an off-white solid just started to precipitate; the mixture was cooled slowly to 5°. The light tan crystals were dried to give 4.5 g (48%) of the desired product, mp 125–126° (lit.[3] mp 125–126°).

4-*n*-Butylamino-2,5-diamino-6-pyrimidinethiol (VII).—To a gently boiling suspension of 1.7 g (0.005 mole) of IV in 100 ml of water was added with stirring 5.2 g (0.03 mole) of $Na_2S_2O_4$ in small portions during 5 min. The resulting, light yellow solution was heated with stirring for an additional 20 min. After treatment with decolorizing charcoal, the hot filtrate was cooled in an ice bath; light, lemon yellow needles were separated, washed with water, and dried to give 1.05 g (98%) of analytically pure product, mp 174–175°. The compound gradually darkened and became almost black on storage in a closed sample tube, in the absence of light; $\lambda_{max}^{0.1\,N\,NaOH}$ 225 mμ (ε 12,900), 305 (4570); $\lambda_{max}^{0.1\,N\,HCl}$ 235 mμ (ε 19,500), 311 (24,200).

Anal. Calcd for $C_8H_{15}N_5S$: C, 45.03; H, 7.10; N, 32.84; S, 15.03. Found: C, 44.98; H, 7.18; N, 32.80; S, 15.11.

4-*n*-Butylamino-2,5-diamino-6-methoxypyrimidine (VI) was prepared from III by a method similar to that described for the 6-mercapto analog (VII) except that the reaction was carried out in 50% aqueous ethanol. After concentration *in vacuo* and cooling, the analytically pure product separated in 63% yield: mp 112–113.5°; λ_{max}^{EtOH} 286 mμ (ε 8250), 302 (6780).

Anal. Calcd for $C_9H_{17}N_5O$: C, 51.11; H, 8.14; N, 33.18. Found: C, 50.95; H, 7.99; N, 33.38.

4-*n*-Butylamino-2,5-diamino-6-pyrimidinol (X) was prepared from IX by essentially the same procedure as the 6-methoxy analog (VI) except that 70% aqueous ethanol was employed as the solvent at a reaction temperature of 70–75°; yield 83.5%, mp 206–208° dec. While the solid appeared to be indefinitely stable, solutions of the compound darkened in the presence of air, especially on warming. This color change could be reversed by adding a small amount of $Na_2S_2O_4$ or $NaHSO_3$ to the dark solution (stirring). To prepare an analytical sample, the compound was stirred at room temperature with a small volume of ethanol containing some $NaHSO_3$. The saturated, alcoholic solution was then rapidly filtered at room temperature under nitrogen. Upon cooling to −15°, a yellow solid separated which was dried at room temperature *in vacuo*; mp 208–210° dec; λ_{max}^{EtOH} 294 mμ (ε 7190), 367 sh (4880).

Anal. Calcd for $C_8H_{15}N_5O$: C, 48.70; H, 7.68; N, 35.51. Found: C, 48.56; H, 7.49; N, 35.70.

[a] Melting points are uncorrected. All ultraviolet spectra were determined with a Cary 15 spectrophotometer. Elemental analyses are by Galbraith Laboratories, Inc., Knoxville 21, Tenn.

[b] E. Baer and D. Buchner, *J. Biol. Chem.*, **230**, 447 (1958).

N-(2-Chloroethyl)-DL-aspartic Acid and Some Related Amino Acids

JAIME LORES ARNAIZ AND JUANA FALSZPAN

Cátedra de Química Orgánica I, Departamento de Química Orgánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

Received April 12, 1966

Mutagenic effects on Drosophila are shown by many monofunctional alkylating agents.[1] Fahmy and Fahmy[2] pointed out that mutagenicity with the mustards is influenced not only by

(1) W. C. A. Ross, "Biological Alkylating Agents," Butterworth and Co. (Publishers) Ltd., London, 1962, p 65.

(2) (a) O. G. Fahmy and M. J. Fahmy, *J. Genet.*, **54**, 146 (1956); (b) *Genet. Res.*, **1**, 173 (1960); (c) *Heredity*, **15**, 115 (1960); (d) *Genetics*, **46**, 1111 (1961).

TABLE I

N-(2-CHLOROETHYL)-DL-β-AMINOBUTYRIC ACID HYDROCHLORIDE

$$ROOCCH_2CHCH_3$$
$$|$$
$$NHCH_2CH_2X$$

Compd no.	R	X	Yield, %	Mp, °C	Recrystn solvent[a]	Formula	Calcd, % C	H	N	Cl	Found, % C	H	N	Cl
V	H	OH	82	181[b]	A	$C_6H_{13}NO_3$								
VI	CH_3	OH	95	Oil	..	$C_7H_{16}NO_3Cl$			7.09	17.90			6.80	16.70
VII	CH_3	Cl	90	94	B	$C_7H_{15}NO_2Cl_2$	38.80	6.94	6.48	32.87	38.17	7.55	6.56	32.56
VIII	H	Cl	96	Syrup	..	$C_6H_{13}NO_2Cl_2$			6.98	35.10			6.89	34.92

[a] A = dimethylformamide, B = ethyl acetate–acetone (4:1) or acetonitrile. [b] Lit.[3a] mp 181°.

TABLE II

N-(2-CHLOROETHYL)-DL-β-AMINOISOBUTYRIC ACID HYDROCHLORIDE

$$ROOCCHCH_2NHCH_2CH_2X$$
$$|$$
$$CH_3$$

Compd no.	R	X	Yield, %	Mp, °C	Recrystn solvent[a]	Formula	Calcd, % C	H	N	Cl	Found, % C	H	N	Cl
IX[b]	H	OH	46	158	A	$C_6H_{13}NO_3$	48.97	8.84	9.53		48.72	9.03	9.75	
X	CH_3	OH	90	62–64	B	$C_7H_{16}NO_3Cl$	42.53	8.10	7.09	17.90	42.56	8.43	6.79	17.10
XI	CH_3	Cl	92	131–132	C	$C_7H_{15}NO_2Cl_2$	38.80	6.94	6.48	32.87	38.57	7.37	6.44	32.81
XII	H	Cl	96	Syrup	..	$C_6H_{13}NO_2Cl_2$			6.98	35.10			6.92	34.95

[a] A = dimethylformamide, B = acetone, C = ethyl acetate. [b] The starting material was methacrylic acid and a reflux period of 90 min was necessary.

the reactivity of the alkylating groups but also by the molecular configuration of the nonalkylating or "prosthetic" moiety. Three amino acid nitrogen mustards were synthesized for use in the study of the relation of structure to mutagenic activity. None of the compounds tested showed significant activity.

Experimental Section

N-(2-Hydroxyethyl)amino acids were prepared using a slight modification of the literature procedure.[3] Since the N-(2-chloroethyl)amino acids were synthesized by essentially identical experimental procedures, specific data will be given for only one compound; data on the other analogs will be presented in Tables I and II. All melting points (not corrected) were determined in capillaries.

β-Methyl N-(2-Hydroxyethyl)-DL-aspartate (I).—A solution of 9.8 g (0.1 mole) of maleic anhydride in 25 ml of absolute methanol was heated at reflux for 30 min, and the excess methanol was distilled *in vacuo*. The light yellow reaction mixture, after cooling in ice, was treated dropwise (stirring) with 30 ml of ice-cooled pyridine. Then, 6.1 g (0.1 mole) of ethanolamine was added, and the solution was refluxed for 1 min. The solution was left to cool, and the crystalline product was filtered, triturated once in hot acetone and once in methanol, and recrystallized from dimethylformamide giving I (8.75 g, 46%, mp 184°).

Anal. Calcd for $C_7H_{13}NO_5$: C, 43.97; H, 6.80; N, 7.32. Found: C, 44.29; H, 6.87; N, 7.25.

Dimethyl N-(2-Hydroxyethyl)-DL-aspartate Hydrochloride (II).—To 16 ml of cooled (−10°) methanol was added slowly with stirring, 4.76 g (0.02 mole) of purified thionyl chloride, then 3.8 g (0.02 mole) of I. The solution was left at room temperature for 30 min, and the methanol was eliminated under reduced pressure. The evaporation was repeated each time after the addition of three 5-ml portions of methanol and two 8-ml portions of methanol–carbon tetrachloride to afford 5 g of a hygroscopic product, which was dissolved in 10 ml of methanol (Norit), filtered, and precipitated with 30 ml of dry ether. The white product was filtered, washed with 10 ml of ether, and dried (high vacuum, P_2O_5) to yield II (4.15 g, 86%, mp 122°).

Anal. Calcd for $C_8H_{16}NO_5Cl$: C, 39.75; H, 6.62; N, 5.79; Cl, 14.82. Found: C, 39.60; H, 6.87; N, 5.65; Cl, 14.76.

Dimethyl N-(2-Chloroethyl)-DL-aspartate Hydrochloride (III).—To a stirred suspension of 8.45 g (0.035 mole) of II in 30 ml of $CHCl_3$ was added a solution of 8.3 g (0.07 mole) of thionyl chloride in 20 ml of $CHCl_3$. The mixture was stirred at room temperature for 10 min, then at reflux temperature for 40 min, and evaporated *in vacuo* to an oil. The evaporation was repeated after each of three additions of 15-ml portions of $CHCl_3$ and two 10-ml portions of methanol. The crystals were collected, washed with ethyl acetate, and dried. Recrystallization from ethyl acetate–acetonitrile afforded III (8.20 g, 90%, mp 150°).

Anal. Calcd for $C_8H_{15}NO_4Cl_2$: C, 36.92; H, 5.76; N, 5.38; Cl, 27.33. Found: C, 36.48; H, 6.02; N, 5.29; Cl, 27.16.

N-(2-Chloroethyl)-DL-aspartic Acid Hydrochloride (IV).—A solution of 1 g (0.004 mole) of III in 10 ml of concentrated HCl was refluxed for 20 hr. At the end, it was evaporated to dryness under reduced pressure, three times with water and two with benzene, to afford IV, a very hygroscopic syrup which could not be characterized. The yield was essentially quantitative. No suitable solvent for crystallization was found, and no crystalline derivative was obtained, using picric and picrolonic acids and ammonium reineckate.

Anal. Calcd for $C_6H_{11}NO_4Cl_2$: N, 6.08; Cl, 30.87. Found: N, 5.95; Cl, 30.52.

Acknowledgments.—The authors are deeply indebted to Professor Dr. S. Lamdan, Universidad de Buenos Aires, for having suggested the subject of this work, and to Dr. B. B. de Deferrari for the microanalyses.

(3) (a) A. Zilkha and I. Rivlin, *J. Org. Chem.*, **23**, 94 (1958); (b) A. Zilkha and M. D. Bachi, *ibid.*, **24**, 1096 (1959); (c) A. Zilkha, E. S. Rachman, and J. Rivlin, *ibid.*, **26**, 376 (1961).

Some Derivatives of Natural Isoflavones

GIORGIO G. FERRARI AND CESARE CASAGRANDE

Research Laboratories, SIMES S.p.A., Milan (Affori), Italy

Received August 3, 1966

Isoflavones and their glycosides are widely distributed in plants. Some of them have been found responsible for disorders of the female reproductive system in cattle[1] and in experimental studies have shown estrogenic activity.[2] These findings and the structural relationships which may be envisaged between

(1) (a) R. B. Bradbury and D. E. White, *J. Chem. Soc.*, 3447 (1951); (b) J. D. Biggers and D. N. Curnow, *Biochem. J.*, **58**, 278 (1954).

(2) E. W. Cheng, L. Yoder, C. D. Story and W. Burroughs, *Ann. N. Y. Acad. Sci.*, **61**, 652 (1951).