3α-Aminocholestane (2 g., 5.16 mmole.) was refluxed with 15 ml. of formic acid and 10 ml. of acetic anhydride for 12 hr. The solution was cooled, diluted with water, treated with about one half equivalent of 10% sodium hydroxide solution, and extracted with methylene chloride. The methylene chloride solution was washed with water and dried over sodium sulfate. Removal of solvent and crystallization of the residue from acetone gave 1.842 g. (86%) of feathery needles, m.p. 179–181°. Repeated crystallization from acetone gave 3α-formamidocholestane, m.p. 188–189°, $[\alpha]_D^{1.6}$ 35.6° (chf., c, 2.2).

Anal. Calcd. for $C_{28}H_{49}NO$: C, 80.90; H, 11.88; N, 3.37. Found: C, 81.02; H, 11.82; N, 3.27.

3α-Isocyanocholestane. 3α-Formamidocholestane (500 mg., 1.2 mmole.) was covered with 10 ml. of dry pyridine, and 382 mg. (2 mmole.) *p*-toluenesulfonyl chloride was added. The resulting orange solution was allowed to stand for 1.5 hr. at room temperature, cooled, treated with ice, and poured into ice water. The product was extracted with ether, washed twice with water, and dried over sodium sulfate. Removal of solvent and crystallization of the pink residue from acetone gave 442.6 mg. (93%) of flat needles, m.p. 139–141°. Further crystallization from acetone gave 3α-isocyanocholestane, m.p. 141–143°, $[\alpha]_D^{1.6}$ 27° (chf., c, 1.6).

Anal. Calcd. for $C_{28}H_{47}N$: C, 84.56; H, 11.91; N, 3.53. Found: C, 84.67; H, 11.89; N, 3.47.

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Action of Primary Aliphatic Amines on 2-Methyl-1,4-α-naphthopyrone

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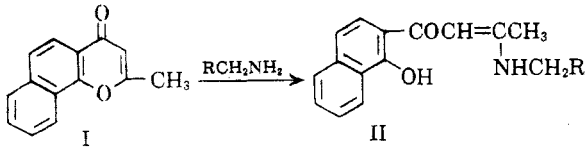
Received February 24, 1958

The action of alcoholic ammonia on 2-methyl-1,4-α-naphthopyrone has been studied by Wittig and Blumenthal.[1] Recently Musante and Stener[2] have studied the action of primary aliphatic amines on the 2-methylchromone derivative Khellin. Both groups of researchers agree that the reaction products are 2-(β-aminocrotonyl)phenols (type II).

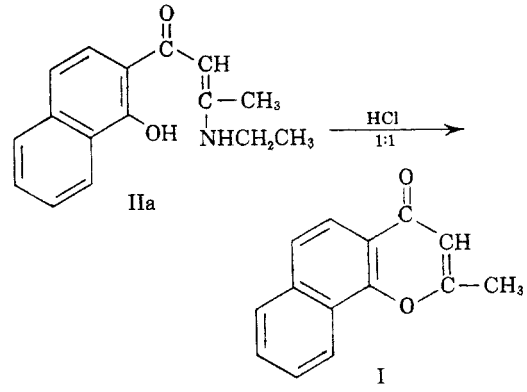
The author has investigated the action of ethylamine, butylamine, and benzylamine on 2-methyl-1,4-α-naphthopyrone(I), and believes that the reaction products are the 2-(β-aminocrotonyl)-1-naphthol derivatives IIa, IIb, and IIc.



I II

a: R = CH_3
b: R = $CH_2CH_2CH_3$
c: R = C_6H_5

The alcoholic solution of these substances gives a green color with alcoholic ferric chloride solution. This fact indicates that they contain a free phenolic hydroxyl group. When IIa was refluxed with dilute hydrochloric acid (1:1), 2-methyl-1,4-α-naphthopyrone (I) was obtained on cooling. The hydrolysis of IIb with aqueous alkali yielded 1-hydroxy-2-naphthoic acid.



EXPERIMENTAL

2-(β-Ethylaminocrotonyl)-1-naphthol IIa. 2-Methyl-1,4-α-naphthopyrone[3] (2 g.) was heated under reflux with two ml. of ethylamine solution in 20 ml. of ethyl alcohol on a steam bath for 3 hr. The deep yellow crystalline solid that precipitated on cooling was filtered off and crystallized from petroleum ether (b.p. 80–100°); m.p. 126°; yield 1.9 g. It has a green fluorescence and is insoluble in aqueous sodium hydroxide solution (10%). It gives a green color with alcoholic ferric chloride solution and a red color with concentrated sulfuric acid.

Anal. Calcd. for $C_{16}H_{17}NO_2$: C, 75.3; H, 6.7; N, 5.5. Found: C, 75.2; H, 6.7; N, 5.4.

2-(β-Butylaminocrotonyl)-1-naphthol IIb was obtained from 2-methyl-1,4-α-naphthopyrone (2 g.) and butylamine (2 ml.). This compound was crystallized from petroleum ether (b.p. 80–100°) as deep yellow crystals with green fluorescence, m.p. 106°; yield 1.8 g. It dissolved in concentrated sulfuric acid with an orange color and was insoluble in cold alkali. Its alcoholic solution gave a color reaction with ferric chloride solution (deep green).

Anal. Calcd. for $C_{18}H_{21}NO_2$: C, 76.3; H, 7.4; N, 5.0. Found: C, 76.6; H, 7.5; N, 4.8.

2-(β-Benzylaminocrotonyl)-1-naphthol IIc. The same procedure was followed with benzylamine. This compound was crystallized from petroleum ether (b.p. 80–100°), as deep yellow crystals with green fluorescence, m.p. 136°; yield 80%. It gives a red-orange color on treatment with concentrated sulfuric acid and a deep green color with ferric chloride solution.

Anal. Calcd. for $C_{21}H_{19}NO_2$: C, 79.5; H, 6.0; N, 4.4. Found: C, 79.6; H, 6.1; N, 4.5.

Action of hydrochloric acid on IIa. Half a gram of IIa was heated under reflux with 25 ml. of dilute hydrochloric acid (1:1) for 0.5 hr. The colorless crystalline solid that separated on cooling was filtered off and proved, by melting point and mixture melting point (178°), and the deep violet color reaction[4] on adding alkali to its solution in dioxane containing *m*-dinitrobenzene, to be 2-methyl-1,4-α-naphthopyrone.

Hydrolysis of IIb *with alkali.* IIb (0.5 g.) and aqueous sodium hydroxide solution (5%, 25 ml.) was heated under reflux for 2 hr. The filtrate was acidified with dilute hydro-

(1) G. Wittig and H. Blumenthal, *Ber.*, **60**, 1085 (1927).
(2) C. Musante and A. Stener, *Gazz. chim. ital.*, **86**, 297 (1956).
(3) G. Wittig, Fr. Bengert, and H. E. Richter, *Ann.*, **446**, 155 (1926).
(4) A. Schönberg and M. M. Sidky, *J. Org. Chem.*, **21**, 476 (1956).

chloric acid. The precipitate was filtered off and identified as 1-hydroxy-2-naphthoic acid (m.p. and mixture m.p. 190°) and the green color with alcoholic ferric chloride solution).

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Synthesis of Some α- and β-(6-Purinylthio)carboxylic Acids

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Received February 25, 1958

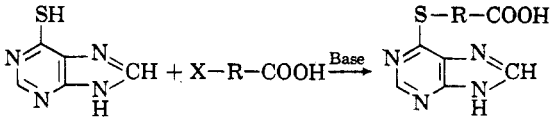
A number of 6-alkyl- and 6-(ω-phenylalkyl)-thiopurines have been found to possess biological activity (in several assay systems) comparable to the corresponding 6-(substituted)aminopurine analogs.[1,2] The unexpected biological activity of the thiopurine derivatives led to the preparation of α-(6-purinylthio)succinic acid[1] as an analog of *N*-(6-purinyl)aspartic acid.[3] Subsequently, the thiosuccinic acid derivative was found to promote growth of etiolated bean leaf disks either in the presence or absence of light.[4] In an effort to extend this latter study, additional purinylthiocarboxylic acid derivatives were prepared and their biological activity on bean leaf expansion will be reported elsewhere.

The desired compounds were synthesized by a condensation of the appropriate α or β-halocarboxylic acid with 6-mercaptopurine under alkaline conditions, using either an equivalent amount of dilute sodium hydroxide or an excess of triethylamine as indicated in Table I. Although the reactants did condense slowly at room temperature in most instances, several of the reactions were carried out in a glass-lined steel bomb heated to about 90°. The sodium hydroxide condensation mixtures, upon acidification, yielded a precipitate which was normally dissolved in alkali and reprecipitated with acid to yield a purified product. In the triethylamine condensation, the reaction mixture was reduced to dryness *in vacuo* in order to remove the excess amine, prior to crystallization.

As in the case of α-(6-purinylthio)succinic acid, the various purinylthiocarboxylic acids indicated in Table I showed little or no response in several biological systems which did respond to 6-alkylthio- and 6-alkylaminopurines. α-(6-Purinylthio)acetic acid at concentration levels as high as 1 mg./ml. did not inhibit hydra tentacle regeneration.[5,6] The purinylthiocarboxylic acids also did not possess either inhibitory or stimulatory effects on a pteridine-inhibited *Lactobacillus arabinosus* at concentration levels up to 40 γ/disk.[7] The presence of a carboxylic acid moiety in the alkyl group of 6-alkylthiopurines appears to cause a loss of biological activity of the compounds in many of these test systems. A decrease in biological activity was also

TABLE I

α- AND β-(6-PURINYLTHIO)CARBOXYLIC ACIDS



Halogen Acid Used	Reaction Conditions	Yield, %	M.p., °C.	Empirical Formula	Analysis N (Calcd.)	Analysis N (Found)
α-Bromoacetic	NaOH, 25°	93[a]	235–260(dec.)	$C_7H_6N_4O_2S$[c]	26.65	26.81
α-Bromopropionic	Triethylamine, 25°	59[a]	199–203	$C_8H_8N_4O_2S$	24.99	24.91
β-Bromopropionic	NaOH, 90°	55[a]	219–220	$C_8H_8N_4O_2S$	24.99	25.01
α-Bromobutyric	Triethylamine, 25°	32[a]	208–209	$C_9H_{10}N_4O_2S$	23.52	23.21
α-Bromovaleric	NaOH, 25°	45[b]	199–205	$C_{10}H_{12}N_4O_2S$	22.21	22.29
α-Bromocaproic	NaOH, 90°	64[b]	178–183	$C_{11}H_{14}N_4O_2S$	21.04	21.06
β-Bromocaproic	Triethylamine, 25°	46[b]	182–184	$C_{11}H_{14}N_4O_2S$[d]	—	—

[a] Recrystallized from water. [b] Recrystallized from ethanol-water. [c] *Anal.* Calcd.: C, 39.99; H, 2.88. Found: C, 40.15; H, 3.25. [d] *Anal.* Calcd.: C, 49.61; H, 5.30. Found: C, 49.33; H, 5.09.

(1) C. G. Skinner, W. Shive, R. G. Ham, D. C. Fitzgerald, Jr., and R. E. Eakin, *J. Am. Chem. Soc.*, **78**, 5097 (1956).
(2) C. G. Skinner, J. R. Claybrook, F. D. Talbert, and W. Shive, *Plant Physiol.*, **32**, 117 (1957).
(3) C. E. Carter and L. H. Cohen, *J. Am. Chem. Soc.*, **77**, 499 (1955).
(4) R. A. Scott, Jr., and J. L. Liverman, *Science*, **126**, 122 (1957).
(5) R. G. Ham, D. C. Fitzgerald, Jr., and R. E. Eakin, *J. Exp. Zool.*, **133**, 559 (1956).
(6) R. G. Ham, Ph.D. dissertation, University of Texas, Austin, June 1957.
(7) E. M. Lansford, Jr., C. G. Skinner, and W. Shive, *Arch. Biochem. Biophys.*, **73**, 191 (1958).