# SYNTHESIS OF N-(6-BENZYLTHIO-9H-9-PURINYL)ACETYLAMINO ACIDS

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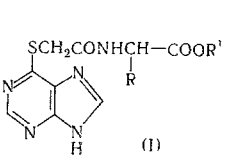
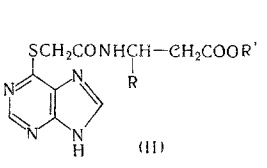
Derivatives of 6-mercaptopurine carrying amino acid residues are of specific interest in the development of possible new antimetabolites for the chemotherapy of cancer. Among amino acids containing the purine residue comparatively few of the compounds which have been described are 6-mercaptopurine derivatives [1-3], and even fewer are compounds which carry the $\alpha$-amino acid substituent at the nitrogen atoms of the purine nucleus.†

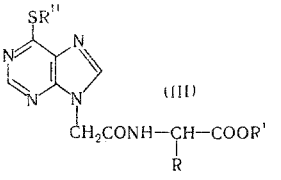
To investigate the effect of the amino acid residue on the pharmacological properties of 6-mercaptopurine it seemed interesting to investigate, in addition to the previously obtained compounds of types (I) and (II) [2] (and also the corresponding dipeptides [3]), amino acids of the type (III), i.e., derivatives of 6-mercaptopurine containing an amino acid residue at N-9 in the imidazole ring:



(I)



(II)



(III)

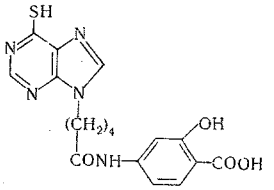
(I) R=$CH_3$, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH_2C_6H_5$; R′=H, $C_2H_5$;
(II) R=$C_6H_5$; R′=H, $C_2H_5$

It was convenient to select the benzyl group to protect the mercapto group, since according to data in [6] the benzyl group at the sulfur atom does not remove activity of 6-mercaptopurine and will probably promote stereoselective alkylation in position 9. Two methods (A and B) were tried for preparation of compounds of type (III).

Method A. The action of ethyl bromoacetate on 6-benzylmercaptopurine in the presence of anhydrous potassium carbonate and in dimethyl sulfoxide (DMSO) gave a 72% yield of ethyl 6-benzylthio-9H-purinylacetate

*Deceased.

†The inhibitor of quinosynthetase succinoadenylate, 4′-(6-mercapto-9-purinyl)valeryl-p-aminosalicylic acid [4],



can be mentioned as a derivative of an aromatic amino acid. Recently [5] $\beta$-(6-mercapto-9-purinyl)-$\alpha$-alanine has been obtained from 6-chloropurine, and $\beta$-(6-methylthio-9-purinyl)-$\alpha$-alanine has been obtained by its alkylation.

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TABLE 1. N-Bromoacetylamino Acid Ethyl Esters

| Original amino acid | From bromoacetyl amino acids (method 1) | | | From amino acid ester hydrochlorides (method 2) | | | Molecular formula | Found, % | | | Calculated, % | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | bromoacetylamino acid taken, g | yield of ester, % | mp, °C, of specimen for analysis (solvent for recrystallization) | ethyl ester hydrochloride taken, g | yield of ester, % | mp, °C (without recrystallization) | | C | H | Br | C | H | Br |
| Glycine | 9,8 | 45 | 74—75 (EA – heptane) | 12,0 | 83* | 74—76 | $C_7H_{10}BrNO_3$ | 32,37<br>32,17 | 4,63<br>4,71 | 35,79<br>35,54 | 32,14 | 4,46 | 35,71 |
| Alanine | 4,95 | 43 | 85,5—86,5 (EA – heptane) | 7,0 | 98 | 78—88 | $C_7H_{12}BrNO_3$ | 35,49<br>35,50 | 5,06<br>5,13 | 33,79<br>33,61 | 35,29 | 5,04 | 33,61 |
| Valine | 4,4 | 75 | — | 7,8 | 79 | — | $C_9H_{16}BrNO_3$ | 40,33<br>40,32 | 6,00<br>6,08 | 30,18<br>29,99 | 40,60 | 6,02 | 30,18 |
| L-Valine | — | — | 46—47† (hexane) | 13,3 | 82 | 74—49 | $C_9H_{16}BrNO_3$ | 40,91<br>40,98 | 5,96<br>6,01 | 29,88<br>29,88 | 40,60 | 6,02 | 30,18 |
| Leucine | 11,16 | 68 | — | 8,4 | 95,6 | — | $C_{10}H_{18}BrNO_3 \cdot {}^1/_2H_2O$ | 41,60<br>41,84 | 6,58<br>6,49 | 27,81<br>27,89 | 41,52 | 6,57 | 27,68 |
| Phenylalanine | 7,15 | 62 | 76—76,5 (ether – heptane, 1 : 1) | 9,9 | 96 | 77—78,5 | $C_{13}H_{16}BrNO_3$ | 49,56<br>49,84 | 4,96<br>4,93 | 25,50<br>25,66 | 49,68 | 5,10 | 25,48 |
| Sarcolysine | — | — | — | 11,0 | 72 ‡ | 96—98 | $C_{17}H_{23}BrCl_2N_2O_3$ | 45,66<br>45,62 | 5,01<br>5,10 | 33,23<br>33,23<br>(Br + Cl) | 44,93 | 5,07 | 33,26<br>(Br + Cl) |

* Also obtained with 63% yield from N-bromoacetylglycine by the method described for methyl esters of acylamino acids in [12].

† $[\alpha]_D^{18} = -30.4°$ (C 1.0, alcohol).

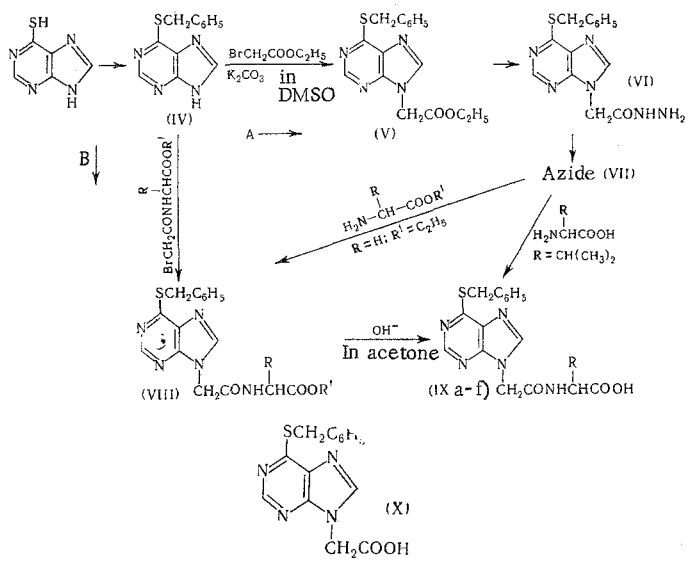
‡ In addition, a further 1 g mixture with the original hydrochloride was obtained (not separated before the end).

TABLE 2. N-Bromoacetylamino Acid Benzyl Esters

| Original amino acid | Bromoacetylamino acid taken, g | Yield of benzyl ester, % | Mp of specimen for analysis, °C | Molecular formula | Found, % | | | Calculated, % | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | C | H | Br | C | H | Br |
| Glycine | 9,0 | 72 | 69—72 ether – heptane | $C_{11}H_{12}BrNO_3$ | 46,15<br>46,33 | 4,36<br>4,34 | 27,62<br>27,70 | 46,15 | 4,19 | 27,97 |
| Alanine | 4,0 | 74 | 57—59 ether – petroleum ether | $C_{12}H_{14}BrNO_3$ | 48,23<br>47,93 | 4,64<br>4,59 | 26,91<br>26,50 | 48,00 | 4,67 | 26,67 |
| Valine | 9,52 | 25 | 62—63 ether – heptane | $C_{14}H_{18}BrNO_3$ | 51,33<br>51,31 | 5,80<br>5,73 | 24,36<br>24,18 | 51,22 | 5,79 | 24,39 |
| Leucine | 6,71 | 59 | 71—72 ether | $C_{15}H_{20}BrNO_3$ | 52,70<br>52,62 | 5,84<br>5,94 | 23,46<br>23,58 | 52,63 | 5,85 | 23,39 |
| Phenylalanine* | — | 64 | 70,5—71,5 ether – heptane | $C_{18}H_{18}BrNO_3$ | 57,56<br>57,71 | 4,91<br>4,80 | 20,70<br>20,86 | 57,45 | 4,79 | 21,27 |

* Obtained by method 2 from 4.4 g phenylalanine benzyl ester hydrochloride (mp 187-188°C, prepared from phenylalanine and thionyl chloride in benzyl alcohol with a yield of 83%).

(V). The reaction was first described in 1961 for 6-chloropurine [7] and gave a yield of 30%. The ester (V) was subsequently converted into the hydrazide (VI) (with a yield of 92%) and the azide (VII), and the latter was then combined with the amino acid or its ester without being isolated in the pure form. In this way combination of the azide (VII) with a glycine ester in homogeneous medium (ethyl acetate) gave N-(6-benzylthio-9H-9-purinyl)acetylglycine ethyl ester (VIII, R = H, R' = $C_2H_5$), and combination with valine in heterogeneous medium [water – tetrahydrofuran (THF)] gave N-(6-benzylthio-9H-9-purinyl)acetylvaline (IX, R = $CH(CH_3)_2$), mp 191°C. In the latter case the reaction mixture also contained a second product of acidic nature, 6-benzylthio-9H-9-purinylacetic acid (X), melting at 212°C, which was the product from decomposition of the azide (VII). The poor yields in the azide coupling stage and the number of stages in the process made Method (A) unacceptable in practice.




(VIII a—f)
R'=$C_2H_5$; R=a—H, b—$CH_3$, c—$CH(CH_3)_2$, d—$CH_2CH(CH_3)_2$, e—$CH_2C_6H_5$, f—$CH_2C_3H_4N(CH_2CH_2Cl)_2$
(VIII $a_1$—$e_1$)
R'=$CH_2C_6H_5$

Method B. The second method involved alkylation of 6-benzylthiopurine by N-bromoacetylamino acid esters (also in DMSO in the presence of anhydrous potassium carbonate). The method was tried on the

TABLE 3. N-(6-Benzylthio-9H-9-purinyl)acetylamino Acid Ethyl Esters

| Amino acid | Amount of 6-benzylmercapto-purine, mmole | Heating time, h | Yield of ester, % | Mp of sample for analysis, °C | Molecular formula | Found, % | | | Calculated, % | | | $\lambda_{max}$, nm ($\varepsilon \cdot 10^{-3}$) in alcohol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | C | H | S | C | H | S | |
| Glycine | 7 | 2,5 | 70 | 168—169 alcohol | $C_{18}H_{19}N_5O_3S$ | 56,07<br>56,04 | 5,12<br>5,08 | 8,44<br>8,46 | 56,10 | 4,94 | 8,31 | 285 (20,5); 292 (20,5) |
| Alanine | 6 | 2,5 | 70 | 168—169 alcohol | $C_{19}H_{21}N_5O_3S$ | 57,07<br>56,80 | 5,43<br>5,37 | 7,88<br>7,71 | 57,14 | 5,26 | 8,02 | 285 (23,0); 292 (22,5) |
| Valine* | 12 | 2,5 | 66 | 150—152 alcohol | $C_{21}H_{25}N_5O_3S$ | 58,98<br>59,13 | 6,05<br>6,08 | 7,22<br>7,55 | 59,02 | 5,85 | 7,50 | 285 (22,5); 292 (20,5) |
| L-valine | 20 | 2,0 | 62,5 | 165—166** | $C_{21}H_{25}N_5O_3S$ | 58,77<br>58,53 | 5,74<br>5,87 | 7,20<br>7,47 | 59,02 | 5,85 | 7,50 | |
| Leucine‡ | 10 | 2,0 | 79 | 107—110 EA – heptane | $C_{22}H_{27}N_5O_3S \cdot {}^1/_4H_2O$ | 58,76<br>58,73 | 6,08<br>6,17 | 7,17<br>6,93 | 59,26 | 6,17 | 7,18 | 285 (14,5); 292 (14,5) |
| Phenylalanine | 10 | 2,5 | 75 | 173—174 alcohol | $C_{25}H_{25}N_5O_3S$ | 63,20<br>63,01 | 5,19<br>5,32 | 6,92<br>6,90 | 63,16 | 5,26 | 6,74 | 285 (23,0); 292 (23,0) |
| Sarcolysine | 5 | 2,0 | 63,5 | 152—154 alcohol | $C_{29}H_{32}Cl_2N_6O_3S$ | 56,37<br>56,38 | 5,19<br>5,14 | 5,19<br>5,16<br>Cl 11,47<br>11,41 | 56,59 | 5,20 | 5,20<br>Cl 11,54 | |

* The product was first obtained in the form of an oily residue and dissolved in chloroform; the solution was washed with water, dried, and evaporated under vacuum; the residue was treated several times with benzene, and each time the benzene was distilled and the product crystallized under ether.

† Compound (V) was also isolated with a yield of 13%.

‡ Treated like the preceding product and recrystallized under heptane.

** $[\alpha]_D^{20} = -34°$ (C 0.48).

ethyl and benzyl esters of a series of amino acids and gave 70-80% yields of N-(6-benzylthio-9H-9-purinyl)-acetylamino esters (VIII) in a single stage.* Alkaline saponification of the ethyl esters (VIII, R' = $C_2H_5$) in acetone gave N-(6-benzylthio-9H-9-purinyl)acetylamino acids (IX). The original ethyl and benzyl esters of the bromoacetylamino acids were obtained in two ways: 1) by esterification of bromoacetylamino acids in the presence of sulfuric acid (for ethyl esters) and p-toluenesulfonic acid (for benzyl esters); this method can be used for valine, leucine, and phenylalanine ethyl esters (with yields of 75, 68, and 62%); in the case of glycine and alanine the yields of the esters of the bromoacetyl derivatives are much lower (45 and 43%); 2) by bromoacetylaction of the corresponding amino ester hydrochlorides; this method can evidently be used for all amino acids (yields in Table 1).

## EXPERIMENTAL

6-Benzylmercaptopurine was obtained from 6-mercaptopurine by the method described in [8]. DL-Amino acids and L-valine were used for the work. The amino ester hydrochlorides were obtained by reaction of the amino acids with thionyl chloride in the appropriate alcohol [9]; the following yields were obtained for the ethyl esters: glycine 98.6%, alanine 91%, valine 86.4%, leucine 98%, phenylalanine 98.2%, sarcolysine 92%.

Bromoacetylvaline Ethyl Ester (Method 1). To a solution of 4.4 g anhydrous bromoacetylvaline in 50 ml absolute ethanol was added 2 ml sulfuric acid, and the mixture was boiled for 6 h without access to moisture. The solution was evaporated to one third of its volume and poured into cold water. The product was extracted with ethyl acetate (EA). The extract was washed with sodium bicarbonate solution and with water, dried over magnesium sulfate, and evaporated under vacuum. The bromoacetylvaline ethyl ester was obtained in the form of a light-yellow oil (yield 3.66 g, 75%),† which was brought into reaction with 6-benzylmercaptopurine without further purification. The ethyl esters of other N-bromoacetylamino acids were obtained similarly (Table 1).

N-Bromoacetylalanine Benzyl Ester (by analogy with amino acid benzyl esters in [10]). A mixture of 4.0 g bromoacetylalanine, 28.5 ml benzene, 2.9 ml benzyl alcohol, and 0.29 g p-toluenesulfonic acid was boiled for 2 h with a Dean and Stark tube, cooled, washed with 2 × 10 ml 5% sodium bicarbonate solution and with water, dried over magnesium sulfate and evaporated under vacuum. After washing several times with petroleum ether the oil which remained slowly crystallized; yield 4.2 g (74%), mp 56-58°C. After reprecipitation from ethereal solution by petroleum ether the product melted at 57-59°C.

The benzyl esters of other bromoacetylamino acids were obtained similarly (Table 2).

N-Bromoacetylalanine Ethyl Ester (Method 2) (similar to acylation of glycine ethyl ester hydrochloride EtPrCBrCOCl [11]). To a mixture of 7.0 g alanine ethyl ester hydrochloride (mp 82-83°C), 64 ml benzene, and 64 ml 8% sodium bicarbonate solution with stirring at room temperature were added simultaneously from two dropping funnels: 1) 64 ml 8% sodium bicarbonate solution; 2) a solution of 4.45 ml $BrCH_2COBr$ in 64 ml benzene. The mixture was stirred for 5 h while maintaining the pH at 7-8 by addition of sodium bicarbonate solution and left over night. The mixture was acidified to pH 6.5, with glacial acetic acid, the benzene layer was removed, and the aqueous layer was extracted with benzene. The extracts were combined, washed with water, and dried over magnesium sulfate. After distillation of the benzene a 10.65 g yield (98.2%) of N-bromoacetylalanine ethyl ester, melting at 88-90°C, was obtained.

The ethyl esters of other bromoacetylamino acids (Table 1) and the benzyl ester of N-bromoacetylphenylalanine (Table 2) were obtained similarly.

Reaction of 6-Benzylmercaptopurine with N-Bromoacetylamino Acid Esters. The 6-benzylmercaptopurine was dried over phosphorus pentoxide at 100°C under vacuum; the dimethyl sulfoxide was dried over barium oxide and distilled under vacuum over sodium hydroxide; the bromoacetylamino acid esters were

*The authors did not undertake the isolation of the 7 isomer from the reaction mixture. It is possible that the 7 isomer is formed in small quantities, but the main reaction product is the 9 isomer (VIII), and the pure (VIII) compounds were chromatographically identical. [The benzyl ester of N-(6-benzylthio-9H-9-purinyl)acetylphenylalanine may be an exception.]

†Reaction in ethyl acetate without heating gave a 62% yield (mixture of 0.95 g bromoacetylvaline, 20 ml ethyl acetate, and 0.44 ml 96% sulfuric acid was left for 5 days at room temperature and treated as described above).

TABLE 4. N-(6-Benzylthio-9H-9-purinyl)acetylamino Acid Benzyl Esters (VII $a_1$-$e_1$)

| (VIII) | Amino acid | Amount of 6-benzylmercapto-purine taken, g | Yield of purified benzyl ester, % | Mp of sample for analysis, °C | Molecular formula | Found, % | | | Calculated, % | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | C | H | S | C | H | S |
| $a_1$ | Glycine | 1,94 | 71 | 175—176 (EA) | $C_{23}H_{21}N_5O_3S$ | 61,68<br>61,71 | 4,90<br>4,66 | 6,86<br>7,45 | 61,71 | 4,70 | 7,16 |
| $b_1$ | Alanine | 1,09 | 66 | 163,5—165 (alcohol) | $C_{24}H_{23}N_5O_3S$ | 62,67<br>62,62 | 5,16<br>5,16 | 6,96<br>7,21 | 62,17 | 4,99 | 6,94 |
| $c_1$ | Valine* | 1,45 | 80 | 42—45 (alcohol) | $C_{26}H_{27}N_5O_3S \cdot {}^1/_2C_2H_5OH$ | 63,46<br>63,38 | 5,59<br>5,51 | 6,43<br>6,45 | 63,28 | 5,86 | 6,25 |
| | | | | 118—120 (EA – heptane) | $C_{26}H_{27}N_5O_3S \cdot {}^1/_2H_2O$ | 62,93<br>62,62 | 5,66<br>5,48 | 6,86<br>6,16 | 62,65 | 5,63 | 6,42 |
| $d_1$ | Leucine† | 1,94 | 87 | 83—86 (EA – heptane) | $C_{27}H_{29}N_5O_3S \cdot H_2O$ | 62,04<br>62,38 | 6,12<br>5,98 | 6,30<br>6,12 | 62,19 | 5,95 | 6,14 |
| $e_1$ | Phenylalanine‡ | 1,21 | 70 | 174—175 (EA) | $C_{30}H_{27}N_5O_3S$ | 67,02<br>66,83 | 5,28<br>5,10 | 6,04<br>6,16 | 67,03 | 5,03 | 5,95 |

* The reaction product was crystallized under heptane.

† The compound crystallizes from alcohol with 0.5 $C_2H_5OH$ and from EA – heptane mixture with 0.5 $H_2O$. The two compounds are chromatographically identical.

‡ The reaction product was crystallized under ether. The yield of unpurified product is given. The UV data are similar to those of the ethyl esters. $\lambda_{max}$, nm 285 and 292 ($\varepsilon \cdot 10^{-3} \sim 20$).

dried over phosphorus pentoxide in a desiccator; twice-calcined potassium carbonate was dried at 135°C under vacuum over phosphorus pentoxide. A 1 mmole sample of 6-benzylmercaptopurine was brought into reaction with 10 ml DMSO, 1.5 mmole N-bromoacetylamino acid ester, and 1.1 mmole potassium carbonate. The reaction took 2-2.5 h at 30°C (bath temperature) and 0.5-1 h at room temperature. The end of the reaction was controlled by thin-layer chromatography on silica (development by iodine, 2:1 and 1:1 acetone – heptane, 4:1 and 3:1 chloroform–acetone, and butanol + 1 drop $NH_4OH$ systems).

N-(6-Benzylthio-9H-9-purinyl)acetylalanine Ethyl Ester (VIII b). To a solution of 1.45 g 6-benzyl mercaptopurine in 60 ml dimethyl sulfoxide was added with stirring 2.14 g N-bromoacetylalanine ethyl ester (mp 85-86°C) and 0.91 g (6.6 mmole) potassium carbonate. The mixture was stirred for 2.5 h at 30°C (bath temperature) and for 1 h at room temperature. The reaction mixture was poured on to 60 g crushed ice and left overnight in the cooler. The precipitate which separated was sucked off, washed with cold water, and dried. A 2.42 g yield of a compound melting at 137-143°C was obtained (with an almost quantitative yield). After recrystallization from alcohol a 1.74 g yield (70%) of compound (VIII b), melting at 168-170°C, was obtained; after repeated recrystallization the melting poing was 168-169°C. The esters of the other N-(6-benzylthio-9H-9-purinyl)acetylamino acids (Tables 3 and 4) were obtained similarly. When the reaction was carried out on unpurified N-bromoacetylamino acid esters the solution gave, in addition to the main reaction product, the corresponding ester of 6-benzylthio-9H-9-purinylacetic acid, which was clearly formed on account of bromoacetic ester contained in the N-bromoacetylamino acid ester.

N-(6-Benzylthio-9H-9-purinyl)acetylalanine (IX b). To a solution of 0.82 g N-(6-benzylthio-9H-9-purinyl)acetylalanine ethyl ester (mp 168-169°C) in 80 ml acetone, with cooling in water, was added 40 ml 0.1 N potassium hydroxide solution. The mixture was shaken and left for 40 min at room temperature. It was then cooled, acidified to pH 3 with hydrochloric acid (1:1), and left overnight in the cooler. The precipitate which separated was sucked off. A 0.71 g yield of a compound melting at 237-239°C and 0.04 g compound melting at 228-230°C were obtained (with an overall quantitative yield). After recrystallization from ethanol compound (IX b) melted at 237-238°C. The ethyl esters of other N-(6-benzylthio-9H-9-purinyl)-acetylamino acids were saponified similarly (Table 5).

Ethyl 6-Benzylthio-9H-9-purinylacetate (V) (under conditions of reaction with 6-chloropurine [7]). To a solution of 1.75 g 6-benzylthiopurine in 80 ml DMSO with stirring was added 0.9 ml ethyl bromoacetate followed by 1.09 g potassium carbonate. The mixture was stirred for 2 h at 30°C (bath temperature), cooled, and diluted with cold water. The precipitate which separated was filtered off, washed with water, and dried under vacuum. A 1.71 g yield (72.5%) of compound (V), melting at 130-131°C, was obtained. After recrystallization from ethanol the melting point was 134.5-136°C. Found %: C 58.46, 58.75; H 4.92, 4.89; S 9.69, 10.02. $C_{16}H_{16}N_4O_2S$. Calculated %: C 58.54, H 4.89, S 9.76.

6-Benzylthio-9H-9-purinylacetic Hydrazide (VI). To a solution of 1.9 g compound (V) in 60 ml absolute ethanol was added 0.46 ml 85% hydrazine. The mixture was boiled for 4.5 h on a water bath under a reflux condenser. The precipitate which separated on cooling was sucked off, washed with alcohol, and dried at 110°C. A 1.66 g yield (92%) of hydrazide (VI), melting at 209-210°C, was obtained. After recrystallization from ethanol the product melted at 212-213°C. Found %: N 26.02, 26.27. $C_{14}H_{14}N_3OS$. Calculated %: N 26.01%.

Reaction of 6-Benzylthio-9H-9-purinylacetic Azide with Glycine Ethyl Ester. To a mixture of 0.63 g hydrazide (VI), 3 ml water, and 12 ml ethyl acetate were alternately added, dropwise and with shaking over a period of 15 min at 0°C, solutions of 0.18 g sodium nitrite in the minimum quantity of water and 0.3 ml concentrated hydrochloric acid in 3 ml water, while maintaining the pH at 2. The mixture was kept for a further 15 min at the same temperature and then extracted with cold ethyl acetate. The extract was dried while cooling over magnesium sulfate and filtered. The decomposition point of the azide (45-50°C) was determined in a sample after distillation of the solvent. A solution of 4 mmole glycine ester in anhydrous ethyl acetate was added to the obtained solution of azide (VII) in ethyl acetate, and the mixture was left overnight in the cooler. Evaporation of the solution under vacuum gave a 0.24 g yield of a compound melting at 154-157°C and a 0.06 g yield of a compound melting at 160-163°C; the combined yield was 0.30 g (39%). A 0.25 g yield (32.5%) of N-(6-benzylthio-9H-9-purinyl)acetylglycine ethyl ester (VIIIa), melting at 164-166°C, was obtained after recrystallizing twice from ethanol. Found %: C 56.52, 56.13; H 5.12, 5.14; S 8.68, 8.89.

TABLE 5. N-(6-Benzylthio-9H--9-purinyl)acetylamino Acids

| Amino acid | Amount, ml | | | Yield of acid, % | Mp, °C, of sample for analysis (alcohol) | Molecular formula | Found, % | | | Calculated, % | | | $\lambda_{max}$, nm ($\varepsilon \cdot 10^{-3}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ethyl ester, taken for saponification | 0.1 N NaOH | acetone | | | | C | H | S | C | H | S | in ethanol | in 0.1 N NaOH |
| Glycine | 1,63 | 85 | 85 | 97 | 246—247 | $C_{16}H_{15}N_5O_3S$ | 53,93<br>54,11 | 4,20<br>4,36 | 9,01<br>8,94 | 53,78 | 4,20 | 8,96 | 284 (27,5)<br>292 (27,0) | 286 (18,1)<br>292 (18,1) |
| Alanine | 0,82 | 40 | 80 | 96 | 237—238 | $C_{17}H_{17}N_5O_3S$ | 55,01<br>55,19 | 4,82<br>4,78 | 8,75<br>8,42 | 54,98 | 4,58 | 8,62 | 283 (19,0)<br>292 (19,0) | — |
| Valine | 1,1 | 125 | 125 | 89 | 193—194 * | $C_{19}H_{21}N_5O_3S \cdot {}^1/_2\ H_2O$ | 55,35<br>55,38 | 5,61<br>5,86 | 7,66<br>7,48 | 55,88 | 5,39 | 7,84 | 283 (26,5)<br>292 (26,5) | 286 (15,0)<br>292 (15,0) |
| L-Valine | 5,4 | 252 | 252 | 72 | 212—214 ‡ | $C_{19}H_{21}N_5O_3S$ | 57,28<br>57,28 | 5,47<br>5,45 | 8,07<br>7,91 | 57,14 | 5,26 | 8,02 | | |
| Leucine | 2,0 | 100 | 100 | 60† | 175,5—176,5 | $C_{20}H_{23}N_5O_3S$ | 55,47<br>55,28 | 5,88<br>5,77 | 7,56<br>7,48 | 55,68 | 5,80 | 7,42 | 283 (16,5)<br>292 (16,5) | |
| Phenylalanine | 3,28 | 138 | 360 | 98 | 213—214,5 | $C_{23}H_{21}N_5O_3S \cdot H_2O$ | 59,24<br>59,47 | 4,90<br>4,94 | 7,13<br>7,07 | 59,36 | 4,95 | 6,88 | 283 (26,0)<br>292 (25,0) | |
| Sarcolysine | 4,30 | 140 | 140 | 97 | 190,5—191,5 | $C_{27}H_{23}Cl_2N_6O_3S$ | 55,37<br>55,26 | 4,86<br>4,76 | 5,32<br>5,28<br>Cl 11,79<br>11,70 | 55,20 | 4,77 | 5,45<br>Cl 12,10 | — | — |

* In another experiment the compound was isolated in anhydrous form.

† A 0.25 g quantity of compound (X) was isolated from the mother solutions.

‡ $[\alpha]_D^{20} = -40°$ (C 0.5).

$C_{18}H_{19}N_5O_3S$. Calculated %: C 56.10, H 4.94, S 8.31. A mixed melting test with a sample of compound (VIII a), obtained by method B, gave no melting-point depression.

Reaction of 6-Benzylthio-9H-9-purinylacetic Azide with Valine. To a solution of 0.31 g hydrazide (VI) in 20 ml 90% aqueous THF solution with stirring at −5°C was added 1.1 ml 1 M sodium nitrite solution and 1.6 ml 1 N hydrochloric acid solution to pH 2. The mixture was kept for 5 min at −5°C. A further 0.6 ml hydrochloric acid was then added, and the mixture was kept for 5 min longer. A 0.15 ml sample of triethylamine was added (to pH 7). The obtained mixture was poured at −5°C into a cooled solution of 0.117 g valine in a small quantity of water and 0.14 ml triethylamine. The mixture was left for 30 min at −5°C and then for 2 days at ~0°C. The solution was filtered and evaporated under vacuum until crystallization began. A few drops of acid were added to pH 3, and the mixture was left to crystallize. A 0.26 g yield of a compound melting at 190-195°C was obtained. Recrystallization from alcohol gave two compounds, melting at: 1) 211.5-212.5°C; 2) 197-200°C. Repeated crystallization gave: 1) 6-benzylthio-9H-9-purinylacetic acid (X), melting at 211.5–212.5°C. Found %: C 55.58, 55.96; H 4.07, 4.28; S 10.64, 10.59. $C_{14}H_{12}N_4O_2S$. Calculated %: C 56.00, H 4.00, S 10.70. 2) N-(6-benzylthio-9H-9-purinyl)acetylvaline (IX c), melting at 191°C. Found %: C 56.69, 56.70; H 5.14, 5.07; S 7.77, 8.01. $C_{19}H_{21}N_5O_3S$. Calculated %: C 57.14, H. 5.26, S 8.02. A mixed melting test with a sample of compound (IX c), obtained by method B, gave no melting-point depression.

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## CONCLUSIONS

1. 6-Mercaptopurine derivatives containing amino acid residues at the nitrogen of the imidazole ring (N-9) were obtained.

2. A single-stage method involving the action of the appropriate N-bromoacetylamino acid esters on 6-benzylmercaptopurine (in dimethyl sulfoxide and in the presence of potassium carbonate) was proposed for the preparation of N-(6-benzylthio-9H-9-purinyl)acetylamino esters. Ethyl and benzyl esters of N-(6-benzylthio-9H-9-purinyl)acetylglycine and the corresponding alanine, valine, leucine, and phenylalanine derivatives (VIII a-e and VIII $a_1$-$e_1$) and also the ethyl esters of N-(6-benzylthio-9H-9-purinyl)acetylsarcolysine (VIII f) and the corresponding L-valine derivatives were obtained.

3. The corresponding N-(6-benzylthio-9H-9-purinyl)acetylamino acids (IX a-f) were obtained from the ethyl esters.

4. N-(6- Benzylthio-9H-9-purinyl)acetylvaline (IX c) and N-(6-benzylthio-9H-9-purinyl)acetylglycine ethyl ester were obtained from 6-benzylmercaptopurine by the multirange method A through the ethyl ester, hydrazide, and azide of 6-benzylthio-9H-9-purinylacetic acid. A by-product of the reaction (6-benzylthio-9H-9-purinylacetic acid) was isolated.

5. The ethyl and benzyl esters of N-bromoacetylglycine and the corresponding alanine, valine, leucine, and phenylalanine compounds and the ethyl esters of N-bromoacetylsarcolysine and the corresponding L-valine compounds were obtained as initial products.

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