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SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF ACETYLSALICYLAMINO ACIDS AND PEPTIDES

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Several water-soluble acetylsalicylamino acids and peptides containing neutral and acidic amino acids were synthesized and investigated for anti-inflammatory activity.

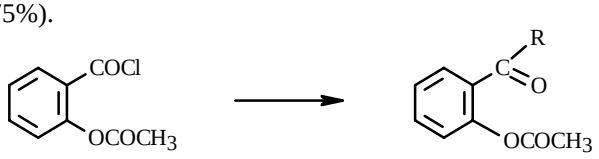
Key words: aspirin, acetylsalicylic acid, amino acids, acid chlorides, *N*-hydroxysuccinimide, peptide.

Side effects of aspirin, acetylsalicylic acid (Ac-Sal-OH), can be avoided by decreasing the acidity and increasing the pharmacological activity, which can be achieved by preparing new amino-acid and peptide derivatives of acetylsalicylic acid [1].

The first attempt to prepare acetylsalicylamino acids was made in 1982 [2].

The goal of the present work was to synthesize water-soluble amino-acid and peptide derivatives of acetylsalicylic acid. We used amino acids with hydrophobic and hydrophilic groups that affected the conformation of the final product in general and its solubility in polar and nonpolar solvents. Therefore, we synthesized and investigated the anti-inflammatory activity of the following compounds: Ac-Sal-Gly-OH (**2**), Ac-Sal-Gly-OMe (**3**), Ac-Sal-Ala-OH (**4**), Ac-Sal-Ala-OMe (**5**), Ac-Sal-Val-OH (**6**), Ac-Sal-Leu-OH (**7**), Ac-Sal-Pro-OH (**8**), Ac-Sal-Phe-OH (**9**), Ac-Sal-Tyr-OH (**10**), Ac-Sal-Glu$(OH)_2$ (**11**), Ac-Sal-Asp$(OH)_2$ (**12**), Ac-Sal-Gly-Gly-OH (**13**), Ac-Sal-Tyr-Pro-OH (**14**), Ac-Sal-Tyr-Pro-Phe-OH (**19**).

The first step in the synthesis was activation of the carboxylic acid of acetylsalicylic acid (**1**) by converting it to acetylsalicylic acid chloride (Ac-Sal-Cl, **1a**) using thionyl chloride and a catalyst of 2-hydroxypyridine (2-HP, 97%) or $AlCl_3$ (81%) and without a catalyst (75%).



1a **2 - 5**

2: R = $NHCH_2COOH$ **4:** R = $NHCH(CH_3)COOH$

3: R = $NHCH_2COOCH_3$ **5:** R = $NHCH(CH_3)COOCH_3$

The highest yield (97%) of crystalline acetylsalicylic acid chloride was obtained using 2-HP catalyst.

Glycine, alanine, and their methyl esters were added to the resulting acetylsalicylic acid chloride.

The methyl esters of acetylsalicylamino acids **3** (78%) and **5** (77%) were prepared in higher yields than acetylsalicylamino acids **2** (48%) and **4** (56%). Obviously this was due to the higher nucleophilicity of the amine group of the amino-acid esters.

The yields of acetylsalicylamino acids were increased by using activated esters. We found that the most effective was the *N*-hydroxysuccinimide ester of acetylsalicylic acid (Ac-Sal-ONSu). The sodium salts of valine, leucine, proline, phenylalanine, tyrosine, glutamic, and aspartic acid were condensed with the *N*-hydroxysuccinimide ester of acetylsalicylic acid by the literature method [3] to give **2** and **4** in increased yields of 84 and 74%, respectively.

Acetylsalicylpeptides were synthesized by a scheme such that the synthesized peptides were easily isolated from the reaction medium with minimal racemization. We used a synthesis of Ac-Sal-Tyr-Pro-Phe-OH (**19**) that clearly demonstrated the possibility in principle of extending the peptide chain stepwise using free terminal amino acids. Part of the Z-Tyr(OBzl)-

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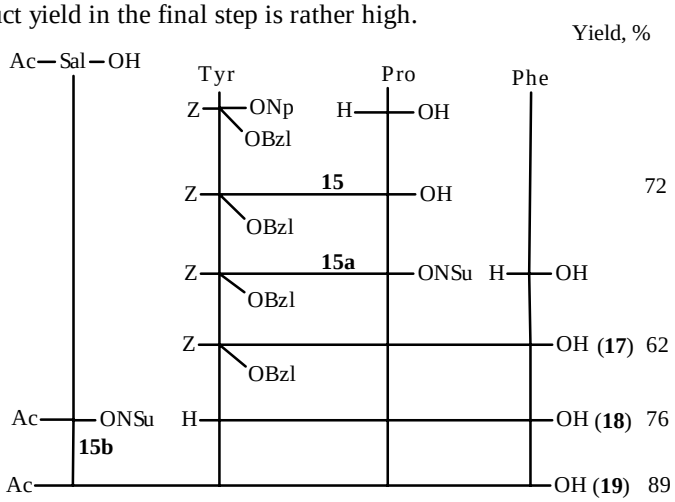
ONp remained unreacted in the synthesis of **15** [4] and was removed using column chromatography over silica gel (L-100/160) with elution by benzene:acetone:acetic acid (100:50:2) and then methanol to isolate the principal product Z-Tyr(OBzl)-Pro-OH (**15**).

The protected tripeptide Z-Tyr(OBzl)-Pro-Phe-OH (**17**) was prepared by condensation of Z-Tyr(OBzl)-Pro-ONSu (**15a**) with phenylalanine in DMF. The condensing agent was *N,N*-dicyclohexylcarbodiimide (DCC). The protected tripeptide **17** was purified by extraction with ethylacetate from an acidified (pH 3-4) aqueous solution of the reaction mixture and subsequent precipitation from ethylacetate by hexane. The Z- and -Bzl groups were removed from the protected tripeptide **17** by catalytic hydrogenation over Pd-catalyst. The final product Ac-Sal-Tyr-Pro-Phe-OH (**19**) was synthesized by condensation of Ac-Sal-ONSu (**15b**) with the sodium salt of H-Tyr-Pro-Phe-OH (**18**) in DMF. Crystalline product **19** was isolated by extraction with ethylacetate from an acidified aqueous solution of the reaction mixture. The synthetic results led to the conclusion that the method using the acid chloride and the *N*-hydroxysuccinimide can be used successfully to synthesize acetylsalicylamino acids and peptides because the product yield in the final step is rather high.



The anti-inflammatory activity of the amino-acid and peptide derivatives of acetylsalicylic acid was determined by screening in rabbits in which inflammation was induced by formalin. The extent of the inflammation was estimated from the amount of leucocytes in the blood as determined on a Picoscale PS-4 instrument.

The results showed that the synthetic derivatives of Ac-Sal-amino acids and peptides have anti-inflammatory activities that differ from each other. However, these properties are clearly evident for the peptide derivatives (**13** and **19**). The anti-inflammatory activity of the synthesized compounds are given below (dose 5 mg/kg):

Compound	*Anti-inflammatory activity, %*
1	100
2	65
4	82
8	56
12	20
13	104
14	94
19	111.

EXPERIMENTAL

We used acetylsalicylic acid prepared by acetylation of salicylic acid and commercial L-amino acids (Reanal, Hungary). Chromatography was performed on Silufol plates (Czech Rep.) using $CHCl_3$:CH_3OH (60:13, A), $CHCl_3$:CH_3OH:CH_3CO_2H (90:10:5, B), C_6H_6:$(CH_3)_2CO$:CH_3CO_2H (100:50:2, C), and C_4H_9OH:CH_3CO_2H:H_2O (3:1:1, D). Elemental analyses were performed on a 240B Perkin—Elmer analyzer; amino-acid analysis, on a Durram D 500 analyzer (USA). Chromatograms were developed using alcoholic ninhydrin (0.5%), I_2 vapor, and Cl_2-benzidine KI. Hydrogenation was carried out in the presence of Pd/C (Merck, 5 and 10 wt.%). Specific optical rotation was determined in a Polamat polarimeter (GDR); melting points, on a Boetius instrument (GDR). Amino-acid analysis was performed on a Hitachi-635 instrument (Japan) after hydrolysis in HCl

(6 N) with phenol (2%) at 105-110°C for 24 h. We used silica gel L-100/160 (Chemapol, Czech Rep.). Solvents were evaporated in a rotary evaporator at 40-50°C (if the temperature is not given).

Acetylsalicylic Acid Chloride (1a). Ac-Sal-OH (**1**, 0.9 g, 5 mmol) prepared as before [5] was dissolved in $CHCl_3$ (6 mL) and treated with $SOCl_2$ (3 mL, 5 mmol) and a catalytic amount of $AlCl_3$. The reaction mixture was heated for 4 h at 80-85°C, cooled, and filtered to remove unreacted Ac-Sal-OH. Solvent was evaporated. The oily product was dissolved in absolute ether, washed with water, dried over Na_2SO_4, and filtered. The filtrate was evaporated to afford **1a** (0.8 g, 81%), mp 58-59°C, R_f 0.86 (B) and 0.76 (D).

The reaction was performed analogously using 2-HP catalyst and without catalyst to give yields of 97 and 75%, respectively.

Ac-Sal-Gly-OH (2). A. A solution of freshly prepared acetylsalicylic acid chloride (**1a**, 2.5 g, 12.5 mmol) in dioxane (20 mL) was stirred, cooled to -5°C to produce a suspension, and treated dropwise over 30 min with glycine (1.22 g, 12.5 mmol) in NaOH (10.2 mL, 12.5 mmol, 1.22 N). The reaction mixture was left overnight at room temperature. Solvent was evaporated to produce an oil that was dissolved in water and acidified with HCl (3 N) until the pH was 3-4. The product was extracted with ethylacetate (3 × 20 mL) and washed with HCl (3 N) and water. Solvent was evaporated to produce an oil that was dissolvd in ether and precipitated with hexane. Repeated reprecipitation from ethylacetate by ether produced **2** (1.5 g, 51%), mp 120-121°C, R_f 0.86 (A) and 0.81 (B).

B. Glycine (0.375 g, 5 mmol) was dissolved in NaOH (4 mL, 5 mmol, 1.25 N). Water was evaporated. The solid was dissolved in DMF, cooled, stirred, and treated with acetylsalicylic acid *N*-hydroxysuccinimide (1.355 g, 5 mmol) [3] in DMF. The reaction mixture was stirred at 0°C for 2 h and left for 1 d. Solvent was evaporated. The solid was dissolved in water and washed with ether (3 × 20 mL). The aqueous layer was acidified with HCl (3 N) until the pH was 3, extracted with ethylacetate (3 × 20 mL), and washed with water until neutral. Solvent was removed. The solid was recrystallized from ethylacetate to afford **2** (0.99 g, 84%), $C_{11}H_{11}NO_5$ (237.11), mp 121-122°C, R_f 0.86 (A) and 0.81 (B). Amino-acid analysis: Gly 1.01(1).

Ac-Sal-Gly-OMe (3). Glycine methyl ester hydrochloride (1.25 g, 10 mmol) prepared as before [6] was dissolved with stirring in $CHCl_3$ (20 mL), cooled to -5°C, and treated with Et_3N (2 g-eq) and dropwise with cooled (-5°C) Ac-Sal-Cl (1.98 g, 10 mmol) in $CHCl_3$ (20 mL). The mixture was stirred for another 1 h and left for 1 d, washed several times with $NaHCO_3$ solution, H_2O, and HCl (0.01 N), washed again with H_2O, dried over Na_2SO_4, and evaporated to afford **3** (oil, 1.9 g, 77%), R_f 0.75 (B) and 0.69 (C).

Ac-Sal-Ala-OH (4). Alanine (0.64 g, 7.2 mmol) was dissolved in NaOH (5.9 mL, 7.2 mmol, 1.22 N) and evaporated to dryness. The solid was dissolved in DMF, cooled, stirred, and treated with the *N*-hydroxysuccinimide ester of acetylsalicylic acid (2.0 g, 7.2 mmol) that was dissolved earlier in DMF. The reaction mixture was stirred at 0°C for 2 h and left for 1 d. Solvent was evaporated. The solid was dissolved in water, washed with ether (3 × 15 mL), acidified with HCl (3 N) until the pH was 3-4, and extracted with ethylacetate (3 × 20 mL). The extract was washed with HCl (3 N) and water until neutral and evaporated to afford an oil that was dissolved in ether, precipitated by hexane, and reprecipitated from ethylacetate by ether. The resulting solid was separated to afford **4** (1.35 g, 74%), $C_{12}H_{13}O_5N$ (251.11), mp 117-119°C, R_f 0.74 (A), 0.81 (B), and 0.69 (C). Amino-acid analysis: Ala 1.0(1).

Ac-Sal-Ala-OMe (5) was prepared analogously to **3** starting with alanine methyl ester hydrochloride (1 g, 7.1 mmol) [6], DMF (20 mL), Et_2N (1 mL), and Ac-Sal-Cl (1.4 g, 7.1 mmol) to afford **5** (1.48 g, 78%), oil, R_f 0.88 (A) and 0.83 (B).

Ac-Sal-Val-OH (6) was prepared analogously to **4** starting with valine (0.84 g, 7.2 mmol) and Ac-Sal-ONSu (2 g, 7.2 mmol). The oily product was reprecipitated from ethylacetate with ether and purified over a column of silica gel (2.6 × 60 cm) with elution by system C to afford **6** (1.36 g, 68%), $C_{14}H_{18}O_5N$ (280.02), mp 184-186°C, R_f 0.70 (A), 0.78 (B), and 0.54 (C). Amino-acid analysis: Val 1.02(1).

Ac-Sal-Leu-OH (7) was prepared analogously to **4** from leucine (0.655 g, 5 mmol) and Ac-Sal-ONSu (1.385 g, 5 mmol) to afford **7** (1.2 g, 73%), $C_{15}H_{18}O_5N$ (295.15), mp 192-194°C, R_f 0.61 (A), 0.72 (B), and 0.65 (C). Amino-acid analysis: Leu 1.0(1).

Ac-Sal-Pro-OH (8) was prepared analogously to **4** from proline (0.632 g, 5 mmol) and Ac-Sal-ONSu (1.385 g, 5 mmol) to afford **8** (1.63 g, 80%), $C_{14}H_{15}O_5N$ (230.14), mp 149-150°C, R_f 0.56 (A), 0.80 (B), and 0.64 (C). Amino-acid analysis: Pro 1.0(1).

Ac-Sal-Phe-OH (9) was prepared analogously to **4** from phenylalanine (0.825 g, 5 mmol) and Ac-Sal-ONSu (1.385 g, 5 mmol) to afford **9** (1.2 g, 69%), $C_{18}H_{16}O_5N$ (279.0), mp 181-183°C, R_f 0.58 (A), 0.71 (B), and 0.65 (C). Amino-acid analysis: Phe 1.01(1).

Ac-Sal-Tyr-OH (10) was prepared analogously to **4** from tyrosine (0.98 g, 5.41 mmol) and Ac-Sal-ONSu (1.5 g, 5.41 mmol) to afford **10** (1.41 g, 76%), $C_{18}H_6O_6N$ (295.04), mp 167-169°C, R_f 0.67 (A), 0.63 (B), and 0.69 (C). Amino-acid analysis: Tyr 1.0(1).

Ac-Sal-Glu(OH)$_2$ (11) was prepared analogously to **4** from glutamic acid (1.470 g, 10 mmol) and Ac-Sal-ONSu (2.77 g, 10 mmol) to afford **11** (2.68 g, 68%), $C_{14}H_{15}O_7N$ (309.14), mp 185-187°C, R_f 0.69 (A), 0.57 (B), and 0.61 (C). Amino-acid analysis: Glu 0.94(1).

Ac-Sal-Asp(OH)$_2$ (12) was prepared analogously to **4** from aspartic acid (0.665 g, 5 mmol) and Ac-Sal-ONSu (1.385 g, 5 mmol) to afford **12** (1.15 g, 78%), $C_{13}H_{13}O_7N$ (295.18), mp 171-172°C, R_f 0.61 (A), 0.25 (B), and 0.20 (C). Amino-acid analysis: Asp 1.03(1).

Ac-Sal-Gly-Gly-OH (13). H-Gly-Gly-OH (0.66 g, 7.2 mmol) [7] was dissolved in NaOH (4.62 mL, 7.2 mmol, 1.08 N), cooled to 0°C, stirred, and treated with Ac-Sal-ONSu (1.38 g, 7.2 mmol) that was dissolved earlier in DMF. The reaction was carried out at 0-1°C for 3 h and left for 1 d. Solvent was evaporated. The solid was dissolved in distilled water (40 mL), washed with ether (3 × 15 mL), acidified with HCl (3 N) until the pH was 3, and extracted with ethylacetate (3 × 20 mL). The ethylacetate extract was washed with HCl (3 N) and water until the washings were neutral. Solvent was evaporated. The solid was reprecipitated from ethylacetate with ether to afford **13** (1.2 g, 82%), $C_{13}H_{14}O_6N_2$ (294.13), mp 210-212°C, R_f 0.59 (A), 0.41 (B), and 0.50 (C). Amino-acid analysis: Gly 2.04(2).

Ac-Sal-Tyr-Pro-OH (14) was prepared analogously to **13** from H-Tyr-Pro-OH (0.08 g, 0.29 mmol) [7] and Ac-Sal-ONSu (0.08 g, 0.29 mmol) to produce a thick mass that was purified over a silica-gel column (3 × 25 cm). Impurities were eluted from the column using system C. The principal product eluted with CH_3OH to afford **14** (0.12 g, 81%), mp 113-115°C, R_f 0.55 (A), 0.37 (B), and 0.41 (C), $[\alpha]_D^{24}$ -16.18° (*c* 1, CH_3OH). Amino-acid analysis: Tyr 1.0(1), Pro 1.02(1).

***Z*-Tyr(OBzl)-Pro-OH (15)** was prepared analogously to **13** from proline (1.00 g, 8.76 mmol) and *Z*-Tyr(OBzl)-ONp (4.62 g, 8.76 mmol) [7] to afford **15** (3.18 g, 72%), $C_{21}H_{22}O_6N_2$ (398.386), mp 68-69°C, R_f 0.86 (B), 0.50 (C), and 0.90 (D), $[\alpha]_D^{24}$ -10.24° (*c* 1, ethanol).

H-Tyr-Pro-OH (16). Compound **15** (0.2 g) was hydrogenated in CH_3OH to afford amorphous **16** (0.08 g, 72%), R_f 0.1 (A) and 0.21 (B).

***Z*-Tyr(OBzl)-Pro-Phe-OH (17)** was prepared analogously to **13** from **15** (2 g, 3.9 mmol) in ethylacetate, *N*-hydroxysuccinimide (0.5 g, 4.34 mmol), DCC (0.89 g, 4.34 mmol), and phenylalanine (0.66 g, 4 mmol) to afford **17** (1.23 g, 62%), mp 76-78°C, R_f 0.96 (A), 0.92 (B), 0.49 (C).

H-Tyr-Pro-Phe-OH (18). Compound **17** (0.2 g, 0.308 mmol) was hydrogenated in CH_3OH to afford **18** (0.1 g, 76%) (amorphous), R_f 0.1 (A) and 0.16 (B).

Ac-Sal-Tyr-Pro-Phe-OH (19) was prepared analogously to **13** from **18** (0.1 g, 0.23 mmol) and Ac-Sal-ONSu (0.063 g, 0.23 mmol). The product was isolated as an oil and treated with water. The emulsion was washed with ether (3 × 20 mL). The aqueous layer was acidified with HCl (3 N) until the pH was 3, extracted with ethylacetate (3 × 20 mL), and washed with HCl (3 N) and water. The ethylacetate was evaporated. The solid was worked up from ethylacetate with ether to afford **19** (0.12 g, 89%), mp 118-120°C, R_f 0.50 (A), 0.33 (B), and 0.38 (C), $[\alpha]_D^{24}$ -28° (*c* 1, CH_3OH). Amino-acid analysis: Tyr 1.0(1), Pro 1.02(1), Phe 1.02.

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