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Some Condensation Products of Helicin. III. Syntheses with Tuberculostatically Active Substances*

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The condensation compounds of helicin and tetraacetylhelicin, respectively, with *p*-aminosalicylic acid (PAS), isoniazid (INH) and thiosemicarbazide were synthesized. The bacteriological investigations of these compounds are described. The compounds with PAS and INH showed a tuberculostatic activity in vitro.

The well known drugs *p*-aminosalicylic acid (PAS) and isonicotinoyl hydrazide or isoniazid (INH), used in the chemotherapy of tuberculosis, have some untoward properties. Their tuberculostatic action decreases with longer taking, because Mycobacterium tuberculosis becomes resistant. Besides that PAS is insoluble in water, so that sodium salt is used, which provokes the ion disturbance in the body when taken in large doses. Several authors prepared different derivatives of the drugs mentioned above in order to improve their solubility in water on one side, and to make longer their inhibition of growth of virulent human tubercle bacilli on the other side. Among the other derivatives they synthesized Schiff bases with various aromatic aldehydes and also with salicylaldehyde[1]. The product of condensation with INH and salicylaldehyde was prepared too[2]. This product, *o*-hydroxybenzal-isonicotinoyl-hydrazon, is known in the therapy under the name of »Nupasal« or »Salizid«[3].

Some authors synthesized PAS with glucose and got substances, which are soluble in water, less toxic and have longer tuberculostatic action[4, 5]. In those compounds glucose is bonded with the OH- or NH_2-group of PAS as *N*-glucoside.

We synthesized condensation-products of PAS and INH with glucoside helicin (salicylaldehyde-β-D-glucopyranoside or 2-oxybenzaldehyde-β-D-glucopyranoside). The helicin was prepared from glucoside salicin by oxidation with nitric acid[6, 7, 8]. We condensed PAS with helicin in water or ethanol solution and with piperidine as the condensing agent. A new watersoluble compound 2-(1-salicylal-4'-aminosalicylic acid)-β-D-glucopyranoside, $C_{20}H_{21}O_9N$ (I) was obtained. The condensation product of PAS and salicylaldehyde is insoluble in water. Our compound of PAS with tetraacetylhelicin, $C_{28}H_{29}O_{13}N$ (II), is also insoluble in water. These compounds, as Schiff bases, have a azomethin-group and are of yellow and brownish-red colour.

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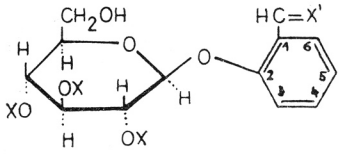

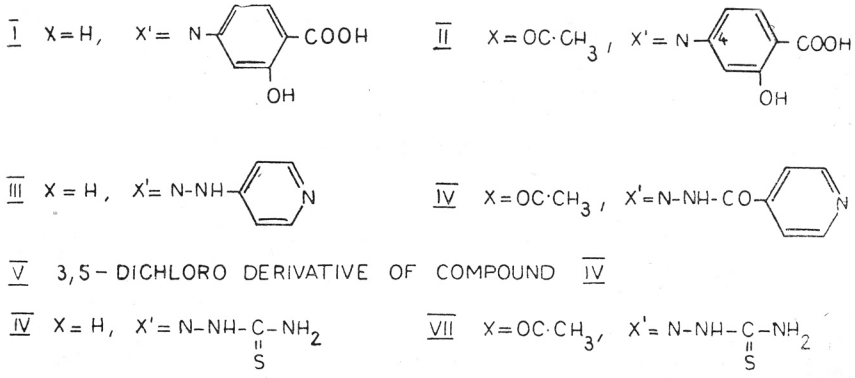

At the beginning of our experiments we found that PAS dissolves easily in a solution of salicin in water (5 g. PAS dissolved in a solution of 1.5 g. salicin in 100 ml. of warm water). This solution has marked tuberculostatic activity in vitro (Table I).

TABLE I

Tuberculostatic activity of different concentrations of PAS dissolved in a solution of 1.86 *g. salicin in* 100 *ml.*

Date	Control	1	10	20	30	40	50	100	250
18. VI. 57.	—	—	—	—	—	—	—	—	—
25. VI.	+	—	—	—	—	—	—	—	—
1. VII.	++	+	—	—	—	—	—	—	—
8. VII.	++	+	+	—	—	—	—	—	—
15. VII.	+++	++	++	—	—	—	—	—	—
28. VII.	+++	+++	++	—	—	—	—	—	—
5. VIII.	+++	+++	++	—	—	—	—	—	—

Mycobact. Tbc in vitro on the Loewenstein-Jensen nutritive substrate[9]: Control = = without PAS; (—) = no bacterial growth; (+), (++) and (+++) increasing degree of bacterial growth. The numbers in the table show the concentration of PAS in γ/ml.

It could be mentioned that salicin alone is an interesting substance in chemotherapy. In the concentration of 20 γ PAS/ml. of the solution there is no bacterial growth after 6 weeks and this is an evidence of the tuberculostatic activity. Pharmacological and toxicological investigations of this solution were made on mice. The solution was well tolerated by mice.

The compound (I) showed a tuberculostatic activity in vitro. The results of bacteriological investigation are shown in Table II.

TABLE II

Tuberculostatic activity from the helicin-PAS compound (I)

Date	Control	1	10	100	110	120	130	140	150	200	250
18. VI. 57.	—	—	—	—	—	—	—	—	—	—	—
25. VI.	+	+	—	—	—	—	—	—	—	—	—
1. VII.	++	++	+	—	—	—	—	—	—	—	—
8. VII.	+++	++	+	+	—	—	—	—	—	—	—
15. VII.	+++	+++	++	++	+	+	+	+	—	—	—
22. VIII.	+++	+++	+++	++	++	+	+	+	+	+	—
29. VII.	+++	+++	+++	+++	++	++	++	++	++	+	+

Concentration of helicin-PAS compound in γ/ml., control without compound (I).

By the reaction of the INH with helicin a water soluble, paleyellowish compound was obtained. Equimolare quantities of substances were dissolved in warm water, and by refrigerating a condensation product of helicin and isoniazid, helicin-isonicotinoyl-hydrazone or 2-(1-salicylal-isonicotinoylhydrazone)-β-D-glucopyranoside, $C_{19}H_{21}O_7N_3$ (III) was crystallized.

Tetraacetylhelicin with INH in absolute alcohol or glacial acetic acid gives a insoluble, colourless compound, $C_{27}H_{29}O_{11}N_3$ (IV). Chlorination of IV in glacial acetic acid solution gave the dichloro derivative, $C_{22}H_{27}O_{11}N_3Cl_2$ (V). We supposed that a substitution product with two chlorine atoms in position 3 and 5 in the benzene ring was obtained. This product is colourless and insoluble in water.

The results of the bacteriological investigation of compound III are shown in Table III. This compound is highly active in inhibiting the growth of tubercle bacilli in vitro.

TABLE III

Tuberculostatic action of helicin-INH compound

Date	Control	0.0187	0.0375	0.075	0.15	0.3	0.6	3	15
11. VII. 57.	—	—	—	—	—	—	—	—	—
18. VII.	+	+	—	—	—	—	—	—	—
25. VII.	++	++	+	+	—	—	—	—	—
1. VIII.	+++	++	++	+	—	—	—	—	—
8. VIII.	+++	+++	++	+	—	—	—	—	—
15. VIII.	+++	+++	+++	++	+	—	—	—	—
22. VIII.	+++	+++	+++	+++	+	—	—	—	—

Concentration of helicin-INH compound in γ/ml., control without compound III.

A strong tuberculostatic action in vitro of compound III was obtained at the concentration of 0,3 γ/ml. (corresponding to the concentration of 1 γ/ml. of INH). This compound did not show any toxic action either. Further bacteriological investigation with this compound would be interesting.

It is known that some thiosemicarbazones with aromatic aldehydes, e. g. 4-acetyl-aminobenzaldehyde-thiosemicarbazone (conteben), possess tuberculostatic activity[10]. The salicylaldehyde-thiosemicarbazone[11] was also synthesized. We therefore prepared the semicarbazone of helicin and tetraacetylhelicin. We obtained helicin-thiosemicarbazone, $C_{14}H_{19}O_6N_3S$ (VI) and tetraacetylhelicin-thiosemicarbazone, $C_{22}H_{27}O_{10}N_3S$ (VII), colourless crystallized compounds, in a very good yield. The compound VI did not show any tuberculostatic action in vitro.

EXPERIMENTAL

Equimolar amounts of substances in water, ethanol or acetic acid solution with the condensing agent were refluxed on a water bath. All melting points were determined with Kofler's heating microscope.

The condensation product of helicin and PAS (I)

0.56 g. of helicin (2 m*M*) were dissolved in 50 ml. of hot water. 0.3 g. (2 m*M*) of PAS and two drops of piperidine were then added. The clear solution was heated for a short time under reflux. A dark red colour was quickly developed. Recrystallization from dilute ethanol yielded 0.55 g. (67%) of brownish platelets, m. p. > 229°C (decomp.). This substance is soluble in warm water.

Anal. 4.660 mg. subst.: 9.773 mg. CO_2, 2.449 mg. H_2O
5.067 mg. subst. : 0.160 ml. N_2 (22°C, 712 mm)
$C_{20}H_{21}O_9N$ (419.4) calc'd: C 57.27; H 5.05; N 3.33%
found: C 57.23; H 5.38; N 3.29%

The condensation product of tetraacetylhelicin and PAS (II)

A solution of tetraacetylhelicin (1.81 g., 4 m*M*) in abs. ethanol and PAS (0.61 g., 4 m*M*) with two drops of piperidine was heated under reflux for 4 hours. After heating it was hot filtered and evaporated to a small volume. A part of the product crystallized in the refrigerator. The product was precipitated by adding water. Recrystallization from aqueous ethanol yielded 1.4 g. (80%). The pale-yellow crystalls, after drying at room-temperature over calcium chloride, had the m. p. of 128°C.

Anal. 6.003 mg. subst.: 11.809 mg. CO_2, 2.847 mg. H_2O
$C_{28}H_{29}O_{13}N \cdot 2H_2O$ (623.55) calc'd: C 53.93; H 5.33%
found: C 53.68; H 5.31%

The substance dried under high vacuum at 60°C is very hygroscopic; m. p. 150°C (decomp.).

Anal. 5.811 mg. subst.: 12.300 mg. CO_2, 2.827 mg. H_2O
5.800 mg. subst.: 0.114 ml. N_2 (20°C, 712 mm)
$C_{28}H_{29}O_{13}N$ (587.4) calc'd: C 58.40; H 4.98; N 2.31%
found: C 57.77; H 5.44; N 2.14%

The condensation product of helicin and INH (III)

A solution of helicin (0.56 g., 8 m*M*) in 10 ml. of hot water and 0.27 g. of the isoniazid with two drops of piperidine was heated under reflux. Recrystallization from water yielded 0.53 g. (67%) of white crystalline product. For the analysis the product was recrystallized from water and dried in vacuum over $CaCl_2$, m. p. 230°C After drying at 60°C in vacuum, m. p. 248°C.

Anal. 5.455 mg. subst.: 10.790 mg. CO_2, 2.714 mg. H_2O
5.880 mg. subst.: 0.525 ml. N_2 (18°C, 710 mm)
$C_{19}H_{21}O_7N_3 \cdot H_2O$ (421.4) calc'd: C 54.13; H 5.49; N 9.97%
found: C 53.93; H 5.59; N 9.54%

The substance is soluble in hot water and alcohol but not very soluble in cold water.

The condensation product of tetraacetylhelicin and INH (IV)

To a solution of tetraacetylhelicin (0.90 g., 2 m*M*) in abs. ethanol or acetic acid (50 ml.), the isoniazid (0.27 g., 2 m*M*) and piperidine (2 drops) were added. The solution was heated for 4 hours under reflux. The crude material was recrystallized from glacial acetic acid with a few drops of water. The yield was 0.95 g. (84%), m. p. 123°C. This substance is insoluble in water and soluble in warm ethanol.

Anal. 5.973 mg. subst.: 11.927 mg. CO_2, 2.660 mg. H_2O
5.460 mg. subst.: 11.059 mg. CO_2, 2.653 mg. H_2O
6.201 mg. subst.: 0.423 ml. N_2 (18°C, 711 mm)
$C_{27}H_{29}O_{11}N_3 \cdot H_2O$ (589.5) calc'd: C 55.00; H 5.30; N 7.12%
found: C 55.25; H 5.44; N 7.38%
C 55.05; H 4.91%

The dichloro derivative of IV, compound (V)

The condensation product IV, salicylal-isonicotinoyl-hydrazone-β-D-glucopyranoside (0.60 g., 2 m*M*) was dissolved in 60 ml. of glacial acetic acid and chlorine passed for 2 hours through the solution at 0°C. After chlorination the mixture was diluted with water and left overnight in closed bottle. The solid precipitate was collected, washed with water and dried. Recrystallization from glacial acetic acid gave 0.55 g. (85%) of white crystalls. Dried in vacuum over calcium chloride, m. p. 150°C.

Anal. 5.738 mg. subst.: 10.637 mg. CO_2, 2.244 mg. H_2O
$C_{22}H_{27}O_{11}N_3Cl_2$ (640.48) calc'd: C 50.63; H 4.25%
found: C 50.59; H 4.38%

The substance is insoluble in water and more soluble in warm than in cold alcohol.

The condensation product of helicin and thiosemicarbazide (VI)

To a solution of helicin (0.28 g., 1 m*M*) in 50 ml. abs. ethanol 0.1 g. thiosemicarbazide and two drops of piperidine were added. The solution was heated for 4 hours under reflux. For analysis it was recrystallized from ethanol and dried in vacuo. The yield was 0.27 g. (71%), m. p. 170°C. The compound is soluble in water.

Anal. 5.048 mg. subst.: 8.300 mg. CO_2. 2.519 mg. H_2O
2.650 mg. subst.: 0.275 ml. N_2 (19°C, 712 mm)
$C_{14}H_{19}O_6N_3S \cdot H_2O$ (375.3) calc'd: C 44.79; H 5.66; N 11.19%
found: C 44.87; H 5.58; N 11.02%

The condensation product of tetraacetylhelicin and thiosemicarbazide (VII)

To a solution of tetraacetylhelicin (0.452 g., 1 m*M*) in 50 ml. abs. ethanol 0.1 g. thiosemicarbazide and two drops of piperidine were added. The solution was heated for 4 hours under reflux, filtered hot and concentrated. Recrystallization from ethanol yielded 0.4 g. (74%) of white crystalls.

Anal. 5.925 mg. subst.: 10.525 mg. CO_2, 3.091 mg. H_2O
$C_{22}H_{27}O_{10}N_3S \cdot H_2O$ (543.5) calc'd: C 48.61; H 5.38%
found: C 48.48; H 5.84%

The compound dried under high vacuum at 70°C is very hygroscopic.

Anal. 5.170 mg. subst.: 0.410 ml. N_2 (21°C, 706 mm)
$C_{22}H_{27}O_{10}N_3S$ (525.5) calc'd: N 8.28%
found: N 8.53%

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IZVOD

O nekim kondenzacionim derivatima helicina. III. Sinteze s tuberkulostatski aktivnim supstancijama

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Pripravili smo kondenzacione produkte helicina (2-oksibenzaldehid-β-D-glukopiranozida) odnosno tetraacetilhelicina sa *p*-aminosalicilnom kiselinom (PAS), hidrazidom izonikotinske kiseline (INH) i tiosemikarbazidom. Dobiveni spojevi helicina otapaju se u vodi, dok se oni s acetilhelicinom ne otapaju. Ispitivana je tuberkulostatska aktivnost dobivenih spojeva in vitro na Löwenstein-Jensenovoj podlozi, pa je nađeno, da su spojevi helicina s *p*-aminosalicilnom kiselinom (I) i hidrazidom izonikotinske kiseline (III) aktivni s obzirom na bacile tuberkuloze, dok spoj s tiosemikarbazidom nije pokazivao aktivnosti. Spojevi s tetraacetilhelicinom u vodi se ne otapaju, pa nije ispitivano njihovo tuberkulostatsko djelovanje.

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