

Derivatives of Isoniazid (I)
Esters of Vanillydeneisonicotinylhydrazide

Isoniazid Türevleri (I)
Vanilidenisonikotinilhidrazid Esterleri

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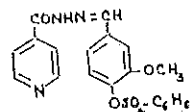
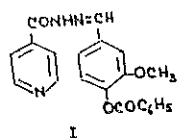
After isoniazid was introduced in the therapy, a lot of isonicotinylhydrazid derivatives were synthesized with the aim to explain the structure-activity relationship and to discover newer tuberculostatics. Among these, arylidene and alkylidene derivatives are the most favourable substances as antituberculous remedies.

Cavallini et al. (1) claimed that isonicotinylhydrazones are almost as active as isoniazid. Bavin et al. (2) and Shchukina et al. (3) confirmed this observation and indicated to the remarkable lower toxicity of hydrazones. Fox and Gibas (4) also showed that alkylidene and arylidene derivatives of isoniazid have better in vivo activity than any other tuberculostatic compounds with the exception of only isoniazid.

The first hydrazones of isoniazid were obtained by Mayer and Mally (5), Cavallini et al. (1,6), Mattu et al. (7), Shchukina et al. (3), Fox and Gibas (4), McMillan et al. (8), Efimowsky and Rumpf (9), Buu-Hoi and Xuong (10-11), Liebermann et al. (12), and many others prepared hydrazones of isoniazid with several aldehydes and ketones.

In this study, we have synthesized two new hydrazones of isoniazid with the esters of vanillin: 3-methoxy-4-benzoyloxybenzylideneisonicotinylhydrazide (I) and 3-methoxy-4-benzenesulfonyloxybenzylideneisonicotinylhydrazide (II), and investigated the analytical characteristics both by classical and spectrophotometric methods.

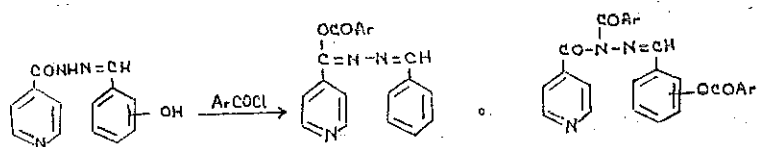
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The main substance of our work, vanillydeneisonicotinylhydrazide was first prepared by Meyer and Mally (5) in 1912 from vanillin and isoniazid. The benzoyl and benzenesulfonyl esters of vanillin were prepared according to the technique described by Wörner (13) and Georgescu (14).

Because of the structural hindrance, it was impossible to synthesize the comp. I and II by direct acylation of vanillydeneisonicotinylhydrazide. As Claisen Haase (15) and Doering and McEven (16) indicated, the pyridine ring may be quaternized by acyl chlorides.

On the other hand, in alkaline media, ester from enolyzed carbonyl and acylated derivative from N' of hydrazide group, may also be formed in addition to phenolic ester :



We have prepared first the benzoyl and benzenesulfonyl esters of vanillin by Schotten-Baumann technique (17, 18). After purification by recrystallization, the esters condensed with isoniazid by heating for 30 minutes in ethanol. The hydrazones which are less soluble than the starting materials precipitated on cooling and crystallized from dilute methanol. The hydrazone forming reaction was easily occurred and there was no remarkable difference between the esterified and unesterified vanillin.

Ferric chloride and ferric ammonium sulfate solutions (19) were used for detection of free phenolic group and 2,3,5-triphenyl-tetrazolium chloride reagent (20, 21) for hydrazide group; no color was developed at the comp. I and II with the reagents mentioned above.

Sodium nitroprusside (22-24), phosphomolibdic acid (22) and potassium dichromate-sulfuric acid reagents were used to detect the hydrazine group.

Pyridine ring was detected by 1-chloro-2,4-dinitrobenzene (25-28); pink-red color obtained, like isoniazid and vanillydeneisonicotinylhydrazide which were used for comparison.

Both hydrazones gave red color on standing for a few minutes with p-dimethylaminobenzaldehyde-HCl reagent as a result of hydrolysis.

The elementary analyses were in accordance to the formulas of the substances.

After hydrolyzing the hydrazones with alkaline hydroxide, the hydrolysis products were identified.

The results of spectrophotometric analyses confirmed the structures of comp. I and II. In UV the absorption maxima which were at 319 and 323 m μ were in accordance with the values given by Grammaticakis as 300-355 m μ for the hydrazones of isoniazid (29) and other acid hydrazides (30, 31). In IR, the characteristic absorption bands indicating aromatic and heterocyclic rings, substitution positions, hydrazide, hydrazone, methoxy and esters groups as well as —SO₂— group for the benzenesulfonyl ester, were observed.

EXPERIMENTAL

3-Methoxy-4-benzoylbenzylideneisonicotinylhydrazide (I)

A mixture of a solution of 2.56 g (0.01 mol) of vanillin benzoate and 1.64 g (0.012 mol) of isoniazid in 50 ml ethanol was refluxed on a water bath for 30 minutes. The reaction product was concentrated and cooled. The precipitate which was formed, was washed first with water, then with a small amount of ethanol and finally with ether, and recrystallized from dilute ethanol, gave 2.35 g (73%). White needles, m.p. 218°; soluble in methanol, ethanol, acetone, dilute acids and alkaline hydroxides; slightly soluble in propylene glycol and glycerol; insoluble in water, ether, petroleum ether and benzene. $\lambda_{\text{max}}^{\text{EtOH}}$ 214 m μ (Σ 24 580), 319 m μ (Σ 23 670). The relation between the absorption and the concentration was linear and obeys the Beer's law for the solution containing 0.18 to 0.92 mg in 100 ml.

It gives a pink-red color with dinitrochlorobenzene; a yellow color with sodium nitroprusside in alkaline media which turns to

bluish green after heating and adding acetic acid; a blue color with phosphomolibdic acid; bluish purple color with cobaltous thiocyanate solution. No colors were developed with ferric chloride, ferric ammonium sulfate and immediately with p-dimethylamino-benzaldehyde-HCl reagents.

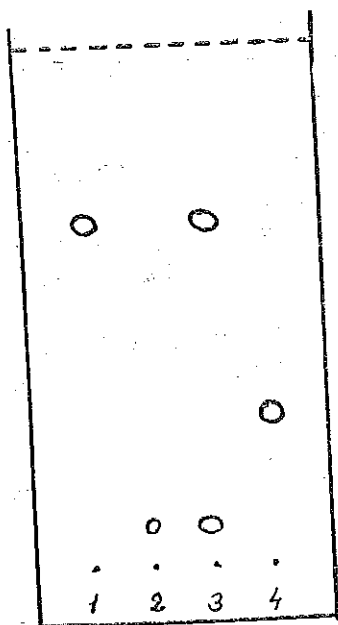


Fig. 1

- 1 — Vanillin benzoate Rf: 0.67
- 2 — INH Rf: 0.08
- 3 — Mixture (1 + 2)
- 4 — Comp. I Rf: 0.28

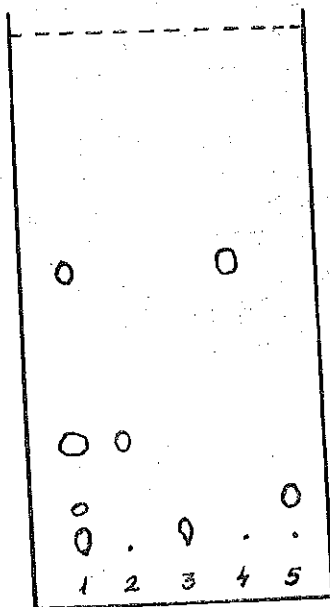


Fig. 2

- 1 — Hydrolysate
- 2 — VINH Rf: 0.20
- 3 — Potassium benzoate Rf: 0.02
- 4 — Vanillin Rf: 0.54
- 5 — INH Rf: 0.08

Thin-layer chromatography on Silica gel HF^{354 + 366} Merck-0.25 mm-gave Rf: 0.28 in methanol-ethyl acetate (10:90) (S₁) (development time: 35 minutes; temperature: 18°C) (Fig. 1) and Rf: 0.22 in chloroform-ethylmethylketone (50:100) (S₂).

Hydrolysis: 0.5 g was hydrolysed with 5 ml of 0.5 N ethanolic potassium hydroxide by heating for 30 minutes under reflux. The hydrolysis product was chromatographed on Silica gel

$\text{HF}_{354+366}$ in S_1 comparatively with vanillin, potassium benzoate, isoniazid (INH) and vanillydene-isonicotinyhydrazide (VINH); four spots, each corresponding to the comparison substances were detected under UV light (Fig. 2).

After addition of water and acidifying with dilute sulfuric acid, the hydrolysate was extracted with ether.

A-The ethereal layer was chromatographed in chloroform-cyclohexan-acetic acid (80: 20: 10) and a spot was found which has the same R_f values of benzoic acid.

After evaporating ether and purifying the residue by sublimation, a substance which melted at 122° was obtained. The mixture with benzoic acid melts at the same temperature.

B-The aqueous layer gave purple color with ferric chloride solutions, VINH and INH were detected chromatographically in system S_1 .

The IR spectra of the compound in KBr was taken by a Perkin-Elmer Spectrophotometer and following absorption bands

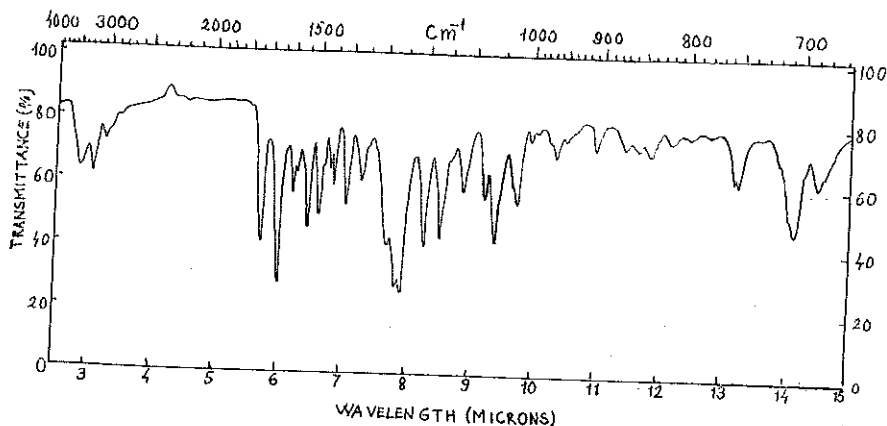


Fig. 3

were observed: 1605 cm^{-1} the —C=C— groups of aromatic and heterocyclic rings; at 1750 , 1260 and 1125 cm^{-1} = ester; at 1675 cm^{-1} C=O ; at 1370 cm^{-1} C=N ; at 2850 , 1175 , 1460 , 1130 and 1030 cm^{-1} —OCH_3 ; at 845 cm^{-1} monosubstituted pyridine; at 758 and 709 cm^{-1} monosubstituted benzene; at 875 and 823 cm^{-1} 1,2,4-trisubstituted benzene (Fig. 3).

Anal, calc. for $C_{21}H_{17}N_3O_4$: C, 67.20; H, 4.53; N, 11.20. Found C, 66.93; H, 4.60; N, 11.31.

3-Methoxy-4-benzenesulfonyloxybenzylideneisonicotinylhydrazide (II).

The compound was obtained from 2.92 g (0.01 mol) of vanillinbenzenesulfonate and 1.64 g (0.012 mol) of isoniazid as described for comp. I. 2.8 g (68%); purified by recrystallization from dilute ethanol.

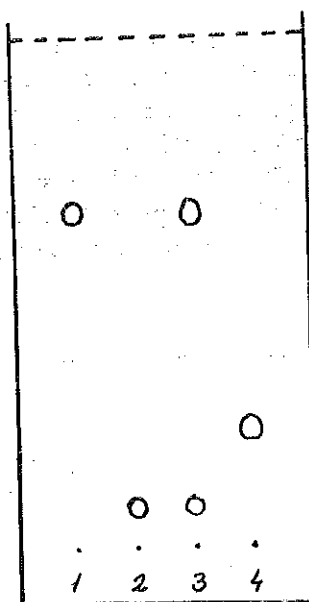


Fig. 4

1 — Vanillin benzen sulfonate Rf: 0.65, 2 — INH Rf: 0.08, 3 — Mixture (1 + 2), 4 — Compound II Rf: 0.22.

White leaflets, m.p. 176° , soluble in methanol, ethanol, acetone, dilute acids and alkaline hydroxides; slightly soluble in propylene glycol and glycerol; insoluble in water, ether, petroleum ether and benzene. $\lambda_{\text{max}}^{\text{EtOH}}$ $323 \text{ m}\mu$ ($\Sigma 27300$). It obeys the Beer's law for the solutions containing 0.27 to 1.27 mg in 100 ml.

It gives a rose-red color with dinitrochlorobenzene; yellow with sodium nitroprusside in alkaline medium which turns to bluish green after heating and adding acetic acid; blue with phosphomolibdic acid; green with sulfo-chromic mixture; bluish-violet with cobaltous thiocyanate solutions. No color reaction was obtained with ferric chloride, ferric ammonium sulfate and immediately with p-dimethylaminobenzaldehyde-HCl reagents.

At TLC the R_f values were 0.22 (Fig. 4) and 0.21 in the solvent systems S_1 and S_2 respectively.

Hydrolysis. After hydrolysis of 0.5 g of substance with potassium hydroxide, four spots were obtained by thin layer chromatography.

Ether was added on the hydrolysate, the precipitate which was formed was washed first with a small amount of ethanol, than with ether. The precipitate did not melt up to 300° . It was fused with potassium hydroxide, dissolved in 1 ml of water and extracted with ether after acidifying with dilute sulfuric acid. The extract gave a spot corresponding to phenol. When plate were sprayed with a 1% solution of ferric chloride the substance was visualized as purple colored spots.

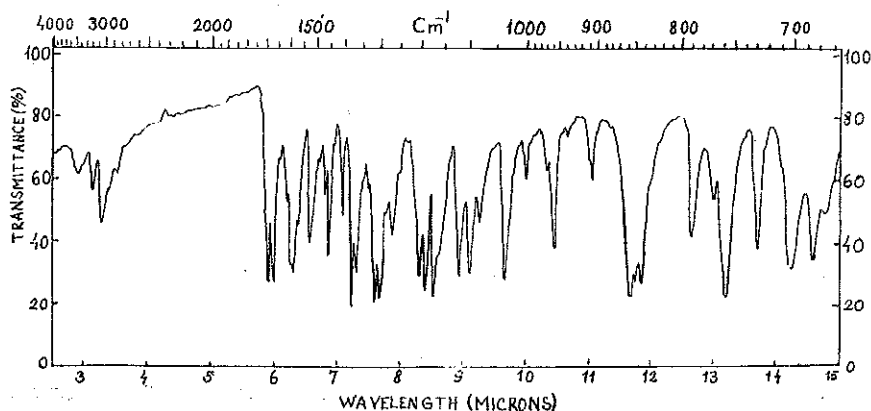


Fig. 5

In the IR spectra of substance (II) in KBr, following absorption bands were obtained: at 1605 cm^{-1} —C=C— groups of aromatic and heterocyclic rings; at 1379 , 1190 and 1170 cm^{-1} sulfonic acid

ester; at 3220 and 1515 cm^{-1} N-H, at 1665 cm^{-1} C=O, at 1330 cm^{-1} C-N groups of hydrazide; at 1661 cm^{-1} —C=N— group; at 2850, 1117, 1460, 1098 and 1035 cm^{-1} —OCH₃; at 845 cm^{-1} monosubstituted pyridine; at 790 and 703 cm^{-1} monosubstituted benzene; at 857 cm^{-1} 1,2,4-trisubstituted benzene (Fig. 5).

Anal. calc. for C₂₀H₁₇N₃O₅ S: C, 58.39; H, 4.13; N, 10.21; S, 7.78, Found C, 58.21; H, 4.03; N, 10.35; S, 7.73.

SUMMARY

Two new isonicotinyldrazones, 3-methoxy-4-benzoyloxybenzylideneisonicotinyldrazide (I) and 3-methoxy-4-benzenesulfonyloxybenzylideneisonicotinyldrazide (II) were synthesized by condensing isoniazid in ethanolic medium with the phenolic esters of vanillin which were previously prepared according to Schotten-Baumann technique. The analytical characteristics of them were investigated by classical and spectrophotometric methods; in thin-layer chromatography, they gave different R_f values than those of the starting materials. Besides of the results of the elementary analyses, UV and IR spectra which confirmed the structure.

ÖZET

İsoniazidi, vanillinin önceden Schotten-Baumann tekniği ile hazırlanmış fenolik esterleri ile etanolü vasatta kondanse etmek suretiyle iki yeni isonikotinihidrazon, 3-metoksi-4-benzoiloksibenzilidenisonikotinihidrazid (I) ve 3-metoksi-4-benzensulfoniloksibenzilidenisonikotinihidrazid (II) elde edildi. Bunların analitik karakteristikleri klâsik ve spektrofotometrik metotlarla incelendi. Hidrazonlar ince tabaka kromatografisinde, hareket maddelerinden farklı R_f değerleri vermekte, ayrıca elemanter analiz neticeleri, UV ve IR spektrumları maddelerin formüllerini teyid etmektedir.

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