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# The Application of Aryl Substituted Derivatives of Xylose as Environmentally Friendly Multipurpose Pesticides

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**Abstract**—A series of aryl substituted derivatives of xylose have been synthesized. Biological tests have revealed high fungicidal activity of the resulting compounds against various phythopathogenic fungi. Additional biological studies have demonstrated high plant growth regulatory activity of the compounds synthesized.

**Keywords:** xylose, aryl substituted derivatives, fungicidal activity, plant growth regulatory activity

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## INTRODUCTION

Pesticides are most commonly used for crop protection against diseases caused by phytopathogenic fungi and bacteria and for insect destruction and also are extensively applied as plant growth regulators [1–3]. They belong to different classes of chemical compounds, with the chemistry and technology of chemical plant protection products being a constantly developing field [4–5]. Over 2500 patents suggesting pesticidal application for compounds obtained by both inorganic and organic chemistry methods are published globally every year, and over 200000 chemicals are tested annually for different pesticidal activity profiles [6–8]. However, crop protection chemicals include negligible number of carbohydrates, in particular functionally substituted monosaccharides [6, 9–14]. In this context, our intention was to prepare practically significant monosaccharides that would produce a dual effect of a fungicide with respect to plant pathogens and of a plant growth regulator. The plant growth regulator activity is ensured by the ability of agricultural plants to take carbohydrates as a source of carbon nutrition for increasing their green mass. As a result, such potential pesticides will not only afford a multipurpose positive effect but also will be almost completely spent without formation of byproducts that need to be disposed of, thereby promoting environmental safety.

The aim of this study was to obtain aryl substituted derivatives of xylose and examine their fungicidal and growth regulatory activities.

## EXPERIMENTAL

**Chemical part.** We used chemicals available from Sigma-Aldrich (US) or Fluka (Switzerland) without further purification. Organic solvents were purified before use by the techniques from [15].

The $^1$H and $^{13}$C NMR spectra were obtained on a Bruker Avance III (FRG) instrument (600 MHz frequency) from 10–15% solutions in $D_2O$ or in MeOH-$d_4$, with TMS as internal standard. MALDI-TOF mass spectra were measured on a MALDI Micromass (US) spectrometer with α-cyano-4-hydroxycinnamic acid matrix. The IR spectra were recorded on a Bruker vector 22 (FRG) from samples pressed into KBr pellets. To monitor the progress of the reaction and identity of the compounds obtained we used thin-layer chromatography on Silica Gel 60 $F_{254}$ (0.25 mm, Merck, FRG) plates in different solvent systems: ethyl acetate–hexane, 1: 1; ethyl acetate–hexane, 3 : 2; and methanol–chloroform, 1 : 9. Substances were detected in the chromatograms using a special developing solution containing 120 g of ammonium molybdate $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ and 5 g of cerium(IV) ammonium nitrate $(NH_4)_2Ce(NO_3)_6$ in 10% sulfuric acid $H_2SO_4$.

Silica Gel 60 (63–200 mm, Merck, FRG) was used as sorbent. The melting point (decomposition temperature) was determined on an Electrothermal IA9300 (UK) instrument.

**Biological part.** *Determination of the fungicidal activity against test cultures.* The substances synthesized were tested on the *Fusarium moniliforme* and *Rhizoctonia solani* (root rot and wilting pathogens of plants) and *Venturia inaequalis* (apple scab pathogen) phytopathogenic fungi using a potato-agar medium into which the dissolved compound at a 0.3% concentration was injected. After 17–20 h the agar plate was inoculated with mycelium of the phytopathogenic fungi. Fungal colonies were counted on day 3 by measuring the diameter of the fungal colony and calculating the percentage inhibition of fungal growth by the formula:

$$\mathrm{PIFG} = \frac{\mathrm{FCDC} \cdot \mathrm{FCDT}}{\mathrm{FCDC}} \times 100\ \%,$$

where PIFG is the percentage inhibition of fungal growth compared to the control, FCDC, fungal colony diameter on the control plate, and FCDT, and fungal colony diameter on the test plate.

The fungicidal activity was tested under glasshouse conditions using green plants: cucumbers, tomatoes, and beans.

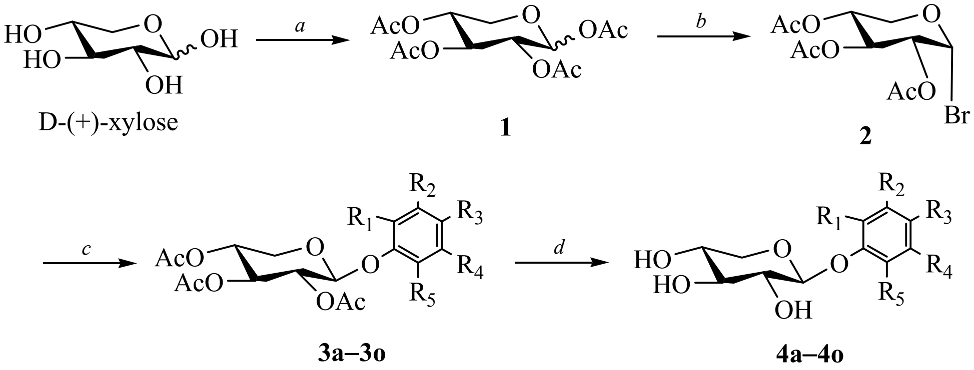
*Determination of the fungicidal activity against plant diseases caused by various phytopathogens.* **Study of the activity against cucumber powdery mildew.** Cucumber plants in the true leaves stage were sprayed with an aqueous suspension of the test compounds at a 0.05% concentration of active material. The control plant was sprayed with water. After drying of the suspension the plants were artificially inoculated with an aqueous suspension of the *Erysiphe cichorociarum* fungus, containing 200 thousand conidia per solution milliliter. The suspension was prepared by washing off the *Erysiphe cichorociarum* conidia from the powdery mildew-infected leaves. The inoculated plants were kept in a glasshouse at 22–30°C. Karathane [2-(1-methylheptyl)-4,6-dinitrophenyl crotonate] [6, 9] served as standard fungicide. The tests were run in triplicate or in quadruplicate. The plant disease development of the cucumbers was assessed on day 10–15.

**Study of the activity against tomato phytophtorosis.** Tomato plants having several leaves were treated with aqueous suspensions containing the tested compounds at a 0.1% concentration. After drying of the suspensions the plants were inoculated with an aqueous suspension of the fungus containing 50 thousand conidia per milliliter of water. The suspension was prepared by growing the *Phytophtora infestans* culture for 5–7 days on potato tuber pieces, from which the conidia were washed off with water. Prior to the inoculation the conidial suspension was kept at 10°C for 20–60 min for swarmer production; the presence of swarmers was checked microscopically. After inoculation the test plants were placed into a moist chamber and exposed for one day at 16–20°C. Then the plants were kept in a glasshouse until the appearance of disease manifestations, typically on day 6–8 after inoculation. As references (standards) served Zineb (zinc *N,N'*-ethylene-bis-dithiocarbamate) [6, 9] or Polycarbacin (zinc ethylene-bis-dithiocarbamate-ethylene thiuram disulfide complex) [6, 9]. The damage was assessed by the presence of characteristic spots on the leave surface.

**Study of the activity against bean gray rot.** Bean plants were grown until the appearance of 6–8 leaves which were sprayed with a suspension of the test compound. The reference (standard) was Difenoconazole {(*cis,trans*-3-chloro-4-[4-methyl-2-(1*H*)-1,2,4-triazol-1-yl-methyl)-1,3-dioxolan-2-yl]phenyl-4-chlorophenyl ether, *cis : trans* isomer ratio, 45 : 55} [9], and the test compound was used at a 0.1% concentration. After drying of the suspension, the treated (for the control, untreated) leaves of the plants were inoculated with a *Botrytis fabae* spore suspension prepared 2 h before the treatment using a 1% glucose solution (containing at least 200 thousand spores per milliliter of solution). Then the test plants were transferred into a moist chamber and kept therein for 72–96 h at 22–23°C and relative humidity of 90%. The damage was assessed on day 4 day after inoculation.

**Determination of acute toxicity of aryl substituted xylose derivatives (4a–4o).** Acute toxicity ($LD_{50}$) of aryl substituted derivatives of xylose **4a–4o** was studied in mongrel white male mice weighing 18–20 g, which were maintained on a standard diet under natural lighting conditions at room temperature. The experimental animals were divided into groups with 10 animals in each group; the observations lasted for 5 days. The tested compounds were suspended in 0.5% aqueous carboxymethyl cellulose solution and injected intraperitoneally. The test results were used for calculating $LD_{50}$ for **4a–4o** by the Kerber method [16, 17]. The pharmacological experiments were carried out in full compliance with the European Convention

**Scheme 1.** Synthesis of aryl substituted derivatives of β-D-xylopyranoside.



Reaction conditions: (*a*) $(CH_3CO)_2O$, $C_5H_5N$, DMAP, 0–20° C, (*b*) HBr (33% $CH_3COOH$), $CH_2Cl_2$, 0°C; (*c*) corresponding substituted phenol, 2,6-lutidine, $Ag_2CO_3$, acetonitrile, 20°C; and (*d*) $NaOCH_3$, $CH_3OH$, 0°C.

$R^1 = R^2 = R^3 = R^4 = R^5 = H$ (**3a, 4a**); $R^1 = R^2 = R^3 = R^5 = H$, $R^4 = NO_2$ (**3b, 4b**); $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = NO_2$ (**3c, 4c**); $R^1 = R^2 = R^5 = H$, $R^3 = R^4 = NO_2$ (**3d, 4d**); $R^1 = R^5 = NO_2$, $R^2 = R^3 = R^4 = H$ (**3e, 4e**); $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = Br$ (**3f, 4f**); $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = F$ (**3g, 4g**); $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = Cl$ (**3h, 4h**); $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = OCH_3$ (**3i, 4i**); $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = OCH_2CH_3$ (**3j, 4j**); $R^1 = R^2 = R^5 = H$, $R^3 = R^4 = CH_3$ (**3k, 4k**); $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = N(CH_3)_2$ (**3l, 4l**); $R^1 = R^3 = R^5 = Cl$, $R^2 = R^4 = H$ (**3m, 4m**); $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = Si(CH_3)_3$ (**3n, 4n**); $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = Si(CH_2CH_3)_3$ (**3o, 4o**).

for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS no. 23, Strasbourg, 18.03.1986) [18].

## RESULTS AND DISCUSSION

Previously, we have synthesized mono- and disaccharides of different classes, containing functional groups S–Hg–R (where R is alkyl or phenyl substituent) at the anomeric carbon atom and studied their fungicidal and herbicidal activities [19]. Biological tests revealed pronounced fungicidal and herbicidal activities in these derivatives, which were even superior in biological activity to the known effective fungicides Delsene [2-(methoxycarbonylamino)benzimidazole], Vectra (bromoconazol), and Rubigan [(±)2,4-dichloro-α-(pyrimidin-5-yl)benzhydryl alcohol)], which are commonly used for controlling phytopathogenic fungi. However, the presence of the mercury atoms in the molecules of the saccharides tested greatly limits their practical use as pesticides. Mercury is known to be concentrated in living organisms and to be able of transforming into highly toxic and carcinogenic methylmercury derivatives, resistant to degradation [20–24]. Hence, despite a high pesticidal value, mercury compounds currently find negligible application in most countries. The development of new pesticides is focused today on the preparation of high-performance, low-toxic, easily degradable in soil, and environmentally friendly compounds [4, 5, 25–28].

In view of the above-said, we synthesized here a series of aryl substituted derivatives of xylose, containing different functional groups in the aromatic moiety, including halogens and nitro(dinitro), alkyl, methoxy, and silyl groups (see Scheme 1).

The first stage consisted of acetylation of D-(+)-xylose with acetic acid anhydride in pyridine, catalyzed by 4-dimethylaminopyridine (DMAP). The resulting xylose tetraacetate **1** was treated with hydrogen bromide (in 33% acetic acid) in anhydrous dichloromethane [29]. The bromination reaction yielded bromo 2,3,4-tri-*O*-acetyl-α-D-xylopyranoside **2** which, because of instability, was introduced without purification in the condensation reactions with various substituted phenols in anhydrous acetonitrile in the presence of 2,6-lutidine (2,6-dimethylpyridine) and silver carbonate ($Ag_2CO_3$) as described in [30–32], which yielded fully protected xylose derivatives, aryl substituted 2,3,4-tri-*O*-acetyl-β-D-xylopyranosides (**3a–3o**). These compounds were treated with sodium methoxide in anhydrous methanol to remove the acetate groups. The resulting deacetylated derivatives were subjected to chromatographic purification with the use of silica gel to give target aryl substituted xylose derivatives **4a–4o**. The $^1H$ and $^{13}C$ NMR spectral parameters and physicochemical charac-

Fungicidal activity of the aryl substituted derivatives of xylose

| Compound | Plant disease development suppression, % | | | Fungal mycelium growth inhibition, % | | |
|---|---|---|---|---|---|---|
| | cucumber powdery mildew | tomato phytophtorosis | bean gray rot | *Fusarium moniliforme* | *Rhizoctonia solani* | *Venturia inaequalis* |
| **4a** | 27 | 19 | 11 | 12 | 10 | 7 |
| **4b** | 39 | 31 | 23 | 54 | 46 | 63 |
| **4c** | 36 | 27 | 29 | 51 | 43 | 60 |
| **4d** | 75 | 64 | 53 | 62 | 57 | 82 |
| **4e** | 70 | 59 | 50 | 71 | 60 | 75 |
| **4f** | 54 | 47 | 42 | 46 | 37 | 54 |
| **4g** | 50 | 41 | 39 | 40 | 45 | 39 |
| **4h** | 61 | 52 | 45 | 56 | 49 | 55 |
| **4i** | 30 | 21 | 17 | 32 | 24 | 21 |
| **4j** | 32 | 20 | 15 | 36 | 29 | 33 |
| **4k** | 29 | 25 | 19 | 32 | 22 | 19 |
| **4l** | 35 | 29 | 23 | 30 | 18 | 28 |
| **4m** | 91 | 82 | 76 | 89 | 100 | 100 |
| **4n** | 34 | 23 | 19 | 24 | 19 | 15 |
| **4o** | 30 | 21 | 15 | 29 | 15 | 11 |

teristics of **4a–4o** were fully consistent with the published data [30–35].

The biological tests showed that the resulting aryl substituted xylose derivatives **4d**, **4e,** and **4m** containing two nitro groups (**4d, 4e)** and three chlorine atoms (**4m**) in the phenyl ring showed high activity against phytopathogenic fungi that cause diseases in agricultural plants (see table). Derivatives **4f, 4g,** and **4h** containing halogens bromine, fluorine, and chlorine, respectively, in the para position of the phenyl ring exhibited moderate fungicidal activity against phytopathogenic fungi. The fungicidal activity of the remaining derivatives, **4a–4c**, **4i–4l**, **4n**, and **4o** was not high, with plant disease development suppressed by no greater than 40% (see table). Derivative **4m** exhibited very high activity against the test cultures of phytopathogenic fungi. For example, its use resulted in 100% suppression of the mycelial growth of *Rhizoctonia solani* (the causal agent of root rot and wilting of plants) and *Venturia inaequalis* (apple scab pathogen), as seen from the table. The fungicidal activity of **4d** and **4e** was also high, ranging from 60 to 82%. The remaining derivatives exhibited from moderate (**4b, 4c,** and **4g–4l**) to low (**4a**, **4n,** and **4o**) fungicidal effect.

Aryl substituted derivatives of β-D-xylopyranoside **4a–4o** were tested as wheat seed protectants against a complex of mold pathogens. It was shown that, in fungicidal activity, **4d, 4e,** and **4m** were slightly inferior to the reference Granosan [*N*-(ethylmercury)-*p*-toluenesulfonanilide] and equal to another reference, Dazomet (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione) [6, 9]. Pharmacological studies of aryl substituted β-D-xylopyranoside derivatives **4a–4o** revealed the acute toxicity ($LD_{50}$) of 770–920 mg/kg (white mice, intraperitoneal injection), which is 30 times lower than that of Granosan (26–30 mg/kg) [9], and 1.5–2.0 times lower than that of Dazomet (450 mg/kg) [9]. However, the use of **4a–4o** under the test conditions was found to reduce seed germination. The remaining derivatives showed moderate (**4b, 4c**, and **4f–4l**) or low (**4a, 4n,** and **4o**) fungicidal activities.

Along with different types of biological activity (fungicidal, antibacterial, insecticidal, etc.), agriculturally applied pesticides displayed a growth regulatory activity in some cases [36–39]. Additional biological tests of derivatives **4a–4o** revealed a growth regulatory activity on the cells cultures of higher plants. The application of these compounds was shown to increase the growth rate of cabbage cell culture and the specific respiration rate by 7–10%, in which parameter they are not inferior to the known plant growth regulators such as Ethephon (2-chloroethylphosphonic acid) [6], NIA-10656 (propylphosphonic acid) [6, 9] and Alar (*N*-dimethylaminosuccinamic acid) [9].

## CONCLUSIONS

(1) Aryl substituted derivatives of xylose have been synthesized with a low toxicity and a high fungicidal activity against a range of phytopathogenic fungi that cause both diseases of agricultural plants and mold development on seeds.

(2) The resulting aryl substituted derivatives of xylose are also suitable as plant growth regulators, which allows them to be considered as environmentally acceptable pesticides for potential multipurpose use.

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