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ORIGINAL ARTICLE

Nitro derivatives from the Arctic ice bacterium *Salegentibacter* sp. isolate T436*

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Twenty-five aromatic nitro, dinitro and trinitro compounds were isolated in low yields of less than 1 mg l^{-1} from a *Salegentibacter* sp. strain T436 derived from Arctic pack ice. Their structures were elucidated by MS and NMR techniques. Seven of these compounds, namely, 2-hydroxy-3-(4′-hydroxy-3′-nitrophenyl)-propionic acid methyl ester (6), 2-chloro-3-(4′-hydroxy-3′-nitrophenyl)propionic acid methyl ester (7), 3-(4′-hydroxy-3′,5′-dinitrophenyl)-propionic acid methyl ester (14), 4′-hydroxy-3′,5′-dinitrophenylethylchloride (16), (4′-hydroxy-3′,5′-dinitrophenyl)-2-chloropropionic acid methyl ester (17), *N*-acetyl-3′,5′-dinitrotyramine (18) and 2,6-dinitro-4-(2′-nitroethenyl)phenol (19) are new, and five are reported in this study from a natural source for the first time.
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Keywords: aromatic nitro compounds; marine bacteria; *Salegentibacter*

INTRODUCTION

During our investigation of secondary metabolites produced by microorganisms in Arctic and Antarctic habitats, we isolated from Arctic pack ice a psychrotolerant, Gram-negative bacterium T436, which was assigned to a distinct group within the genus *Salegentibacter* on the basis of 16S rRNA gene profile and physiological characteristics.[1] Our interest in this strain was attracted by an antimicrobial activity of crude extracts against *Bacillus brevis*, *B. subtilis*, *Nematospora coryli* and *Micrococcus luteus*, as well as by an intensively yellow color.[1] On TLC, numerous yellow zones were visible over the whole polarity range, which were not because of quinones, xanthones, phenazines, polyenes or other common chromophores. On spraying with tin(II) chloride solution, the yellow spots were reduced to colorless or faintly yellow products, which gave intensively yellow or orange Schiff bases on treatment with 4-dimethylaminobenzaldehyde, as an indication of aromatic nitro compounds. This interpretation was confirmed by two IR signals in the range of ν 1510–1570 and 1320–1350 cm^{-1}, and by typical fragments in the EI mass spectra (see Supplementary Figure 1). Nitro compounds are widespread in microorganisms, but are rather rare in nature. Recent examples are nitrobenzyl alcohols from an endophytic mangrove fungus,[2] or nitroresorcinols from myxobacteria.[3]

On TLC, the mononitrophenols isolated in this study absorbed UV light at 254 nm and showed an absorption maximum at $\lambda_{max} \sim 350$ nm in solution (see Supplementary Figure 2). In contrast, the dinitrophenols emitted a yellow UV fluorescence on TLC at 366 nm, and showed a long-wavelength absorption at $\lambda_{max} \sim 430$ nm. A further specialty in the spectroscopic characterization of nitro compounds was with regard to the ^{13}C NMR signals of the quaternary carbon atoms carrying the nitro group, which were only visible at long relaxation delays (1 s).

So far, 25 nitro, dinitro and trinitrophenols were isolated from this strain.[4–6] Compounds **2–6**, **8**, **10**, **11–17**, **23** and **24** were already mentioned in our previous report.[1] In this study, we describe their chemical properties in detail and report additional metabolites obtained on re-fermentation.

RESULTS AND DISCUSSION

Mononitrophenols

For the isolation of further nitro derivatives, a 20 l fermentation conducted under the same conditions as described before[1] was extracted at weakly acidic pH; under basic conditions, the extraction was very ineffective. Chromatographic separation on silica gel, Sephadex LH-20 and by HPLC (Supplementary Figure 3) delivered 10 mononitrophenols. The de-replication of known

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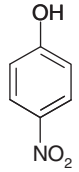
compounds was performed by MS and HRMS, and by comparison with AntiBase data;[7] new compounds were elucidated by additional 2D NMR measurements. *p*-Nitrophenol (**1**),[8] isolated previously from carrot truffle (*Stephanaspora caroticolor*), was easily identified in this manner. It is a widespread environmental contaminant.

A group of nine compounds, **2–10,** showed the characteristic ^{1}H NMR signal pattern of 1,2,4-trisubstituted benzene derivatives. The downfield shift of aromatic ^{1}H NMR signals, the pH-dependent yellow color and the IR data pointed to a group of 4-substituted *o*-nitrophenols with differences in a side chain at C-4. Compounds **1**, **7** and **9** have not been described in our previous report on *Salegentibacter* sp. T436.[1]



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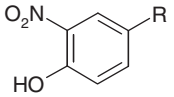


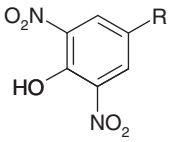
2: R = COOH
3 R = CH_2COOH
4: R = CH_2COOCH_3
5: R = CH_2CH_2COOH
6: R = $CH_2CH(OH)COOMe$
7: R = $CH_2CHClCOOMe$
8: R = CH_2CH_2OH
9: R = CH_2CH_2NHAc
10: R = $CH=CHNO_2$

Beside the signals for a 1,2,4-trisubstituted aromatic ring, the ^{1}H NMR spectrum of **6** revealed an oxymethine multiplet at δ 4.38, a methoxy group at δ 3.70, as well as the AB part of an ABX system at δ 3.10 and 2.98. (−)-APCI mass spectra gave an $[M-H]^-$ ion at *m/z* 240. 2D NMR data revealed the structure of 2-hydroxy-3-(4′-hydroxy-3′-nitrophenyl)propionic acid methyl ester (**6**). The NMR data of 2-chloro-3-(4′-hydroxy-3′-nitrophenyl)-propionic acid methyl ester (**7**) were very similar, but were downfield shifted. Both compounds are reported in this study for the first time. *N*-Acetyl-3′-nitrotyramine (**9**, 0.4 mg l^{-1}) was new in *Salegentibacter* sp. T436, but had been isolated previously from *Pyricularia oryzae*,[9] the causative agent of pyriculariosis, a widespread disease of rice.

Dinitro and trinitro derivatives

Seven simple dinitro compounds **11–17** had already been isolated previously from *Salegentibacter* T436.[1] Two further compounds **18** and **19** have been added now; all are new from natural sources but some had been obtained by synthesis. Their UV maxima (λ_{max} 432 nm) showed a strong bathochromic shift in alkaline solution similar to mononitro-phenols; however, ^{1}H NMR data indicated clearly symmetrically tetrasubstituted benzene derivatives by 2H singlets as the only aromatic signal; the structural differences were again localized in the side chain.

Their structures were derived from 2D NMR measurements and HR mass spectra as 4′-hydroxy-3′,5′-dinitrophenylacetic acid methyl ester (**12**, 0.02 mg l^{-1}), 4′-hydroxy-3′,5′-dinitrophenylacetic acid (**11**),[10] 4′-hydroxy-3′,5′-dinitrophenylpropionic acid (**13**, 0.05 mg l^{-1}), 4′-hydroxy-3′,5′-dinitrophenylpropionic acid methyl ester (**14**)[11] and dinitro-tyrosol (**15**).[12]



11: R = CH_2COOH
12: R = CH_2COOCH_3
13: R = CH_2CH_2COOH
14: R = CH_2CH_2COOMe
15: R = CH_2CH_2OH
16: R = CH_2CH_2Cl
17: R = $CH_2CHClCOOCH_3$
18: R = $CH_2CH_2NHCOCH_3$
19: R = $CH=CHNO_2$

The ^{1}H NMR spectrum of compound **16** (0.03 mg l^{-1}) was similar to that of **13**, although the methylene triplets were at different shifts. The ESI mass spectrum showed molecular ions at *m/z* 246/248 (EIMS) in the ratio of *ca.* 3:1, typical for chlorine. The IR spectrum gave no hints for carboxy or ester groups. From these data and from the empirical formula (by HRMS), the structure of 4′-hydroxy-3′,5′-dinitrophenylethyl chloride (**16**) was derived, which was finally confirmed by heteronuclear multibond correlation (HMBC) data (Supplementary Figure 4). Compound **16** is a new natural product and is also unknown from synthesis.

Compound **17** was obtained in the low yield of 0.04 mg l^{-1}. Its ^{1}H NMR spectrum showed signals of a methine group at δ 4.56, a methoxy group at δ 3.73 and signals of diastereotopic methylene protons at δ 3.18 and 3.06. In the APCI mass spectrum, an $[M-H]^-$ ion at *m/z* 303 indicated chlorine by its isotope pattern. The IR spectrum showed a strong absorption at 701 cm^{-1}, which is characteristic of a C-Cl bond. These data were in agreement with the structure of 4′-hydroxy-3′,5′-dinitrophenyl-2-chloropropionic acid methyl ester (**17**), and HRESIMS confirmed the respective formula, $C_{10}H_9ClN_2O_7$. Compound **17** had not been described before, but the mononitro derivative has been synthesized.[13]

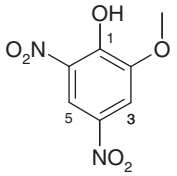
The ^{1}H NMR spectrum of **18** (0.14 mg l^{-1}) resembled that of **16** in the aromatic region, whereas the aliphatic signals corresponded with those of **9**; moreover, the IR spectrum was very similar to that of **9**. The ESI mass spectrum showed an $[M-H]^-$ ion at *m/z* 268. It follows that **18** is the new *N*-acetyl-3′,5′-dinitrotyramine. The structure was further confirmed by HMBC correlations (see Supplementary Figure 5).

The ESI mass spectrum of **19** indicated an odd number of nitrogen atoms by a pseudomolecular ion at *m/z* 254 $[M-H]^-$; HRMS delivered the formula $C_8H_5N_3O_7$. In the ^{1}H NMR spectrum, two 1H doublets of a *trans*-configured double bond appeared at a similar shift as in **10**, which resulted in 2,6-dinitro-4-(2′-nitroethenyl)phenol (**19**); the latter is the first natural trinitro derivative.

Simple dinitro-phenols

A further separation by HPLC yielded two isomeric methoxy-dinitrophenols with the formula $C_7H_6N_2O_6$ (APCI HRMS). Both compounds showed two doublets of *meta*-coupled protons with strong HMBC correlations to the phenolic carbon, but only one proton in both isomers coupled with the carbon connected with the methoxy group). Therefore, in one isomer, the OH group has to be positioned at 1,3-distance with respect to both protons as in **20**, and at 1,2-distance in the other phenol as in **21**. Placing the methoxy group in *para* position to the OH group would result in two symmetrical dinitro compounds and can be excluded: one phenol must therefore be 2-methoxy-4,6-dinitrophenol (**20**) and the other is 3-methoxy-4,5-dinitrophenol (**21**). A direct comparison with synthetic materials[14,15] identified the slightly more polar isomer as **20**. Both assignments were further confirmed by HMBC data. The NMR data of 2-methoxy-3,5-dinitrophenol and 2-methoxy-4,5-dinitrophenol are additionally listed

in the experimental part. It is worth mentioning that the aromatic proton signals of **20** varied up to $\Delta\delta$ 0.2 ($\Delta\delta$ 4 for ^{13}C), depending on the concentration and purity of the sample and the solvent.



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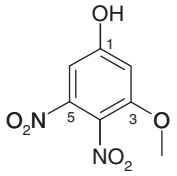

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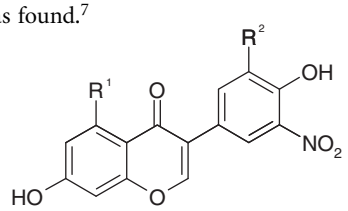
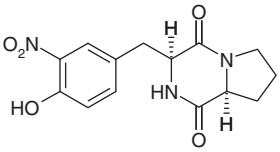
4,6-Dinitroguaiacol (**20**)[16] and isomeric 3,5-dinitroguaiacol[17] are known as metabolites of the red alga *Marginisporum aberrans*; they showed antimicrobial activity against *B. subtilis*. The dinitroresorcinol ether **21** had not been described before.

Additional fermentation products

The isoflavones daidzein and genistein are common by-products in the fermentation of bacteria, if soybean flour or malt extract was used for cultivation. Surprisingly, these compounds were found now in the nitrated form as 3′-nitro-daidzein (**22**, 0.06 mg l^{-1}; see Supplementary Figure 6), 3′-nitrogenistein (**23**) and 3′,5′-dinitro-genistein (**24**, see Supplementary Figure 7). All three compounds had been isolated previously from the genetically engineered *Streptomyces* sp. K_3.[18,19] They showed no significant antimicrobial or phytotoxic activities. In addition, the nitro-diketopiperazine pyriculamide (**25**, 0.3 mg l^{-1}) was found.[7]



22: $R^1 = R^2 = H$
23: $R^1 = OH, R^2 = H$
24: $R^1 = OH, R^2 = NO_2$



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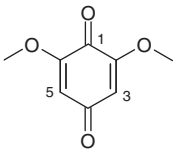

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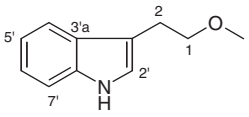

27

A yellow compound with a mass of *m/z* 168 was identified as 2,6-dimethoxy-1,4-benzoquinone (**26**) and was confirmed by literature data. It is known as a plant metabolite with antitumor activity.[20] Metabolite **27** was identified as tryptophol methyl ether, which was known only from synthesis.[21] Its spectroscopical data were in accordance with literature. Further polar UV absorbing fractions contained thymine, uracil and *p*-hydroxybenzoic acid.

ESI fragmentation of aromatic nitro compounds

The MS analysis of nitro compounds was performed best in the negative ESI mode. Under EI conditions, phenolic mononitrocarboxylic acids showed a loss of carbon dioxide and of a 30 Da fragment, a phenomenon that had already been observed in the early 1980s in CIMS experiments[22–25] (see Supplementary Figure 1). This fragmentation may be explained by the reduction of the aromatic nitro group into an amino function in the source during measurement. A better explanation, however, is based on an NO elimination, which was already known from EI experiments.[26] This assumption is further supported by the fact that in the full-scan spectra, no $[M\text{-}H\text{-}30]^-$ ions were detected, whereas these signals appeared after the fragmentation process. In several cases, a signal at $[M\text{-}47\text{-}H]^-$ was observed, which indicates the loss of an HNO_2 molecule.

The dinitro derivatives investigated in this study showed similar fragmentation patterns as those of mononitro compounds. If the nitrophenols contained further leaving groups, such as chlorine atoms, these were eliminated first (in this study as HCl); thereafter, the elimination of NO occurred.

In the positive ESI mode, only those nitro compounds were detectable, which carried a functional group which can be easily protonated, as in **9** or **18**. In these cases, usually an elimination of a 46 Da fragment was observed, which was most likely NO_2.

Biosynthetic considerations

For the biosynthesis of natural nitro groups, at least three different pathways are known: the oxidation of anilines, the direct nitration of phenols with reactive nitrogen species (RNS) and the oxidation of nitroso precursors.

By means of feeding experiments, it was shown that *p*-aminophenylalanine is the precursor of the *p*-nitrophenylserinol part in chloramphenicol,[27,28] as well as of the nitrophenyl unit of aureothin.[29] In the last step of pyrrolnitrin biosynthesis, an amino function is converted into a nitro group by a chloroperoxidase.[30,31] For most of the nitro compounds described in this study, potential amino precursors are, however, not known, and therefore this pathway seems less plausible.

An alternative biosynthetic route to nitro compounds is the oxidation of nitroso precursors,[32] which themselves could be formed with nitrite, NO^+ or $NO^\bullet$ radicals under physiological conditions. Several nitroso compounds related to the nitro compounds isolated in this study were described, for example, 4′-hydroxy-3′-nitrosobenzoic acid from *Streptomyces murayamaensis*.[33] Nitroso compounds such as viridomycin E[34] and others[7] are strong siderophores and are involved in the accumulation of iron, which is a limiting factor for phytoplankton growth in offshore surface waters of the Antarctic[35,36] and northeast Subarctic Pacific Ocean.[37] A search by HPLC/MS in the crude extract of *Salegentibacter* sp. T436 for nitroso compounds corresponding to the nitro derivatives isolated in this study was, however, not successful.

Fermentation under various conditions has shown that nitrate is a precondition for the production of these nitro compounds. As in the brine channel system of the Arctic bottom sea ice increased nitrate concentrations occur,[38] it can be assumed that another pathway is used: the direct nitration by RNS such as peroxynitrite and NO_2, formed as secondary products of NO metabolism in the presence of oxidants including superoxide, hydrogen peroxide and transition metal centres. 3′-Nitrotyrosine formed in such a process has been

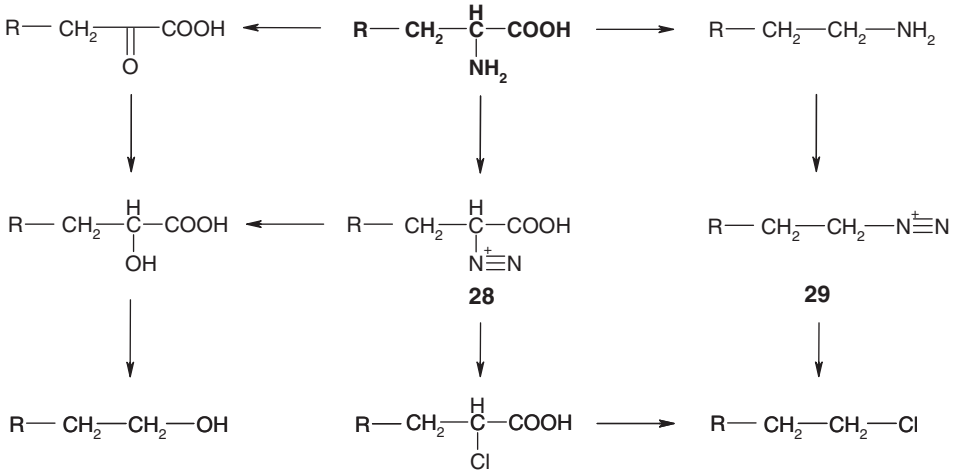

Figure 1 Sidechain transformations in nitrotyrosines by postulated diazonium intermediates **28**/**29**, $R = 4'$-hydroxy-3′-nitrophenyl and 4′-hydroxy-3′,5′-dinitrophenyl, respectively.

discussed as a biomarker of NO-dependent oxidative stress;[39,40] 3′-nitrogenistein is formed under the influence of peroxynitrite,[41] and in the case of dioxapyrrolomycin, such a nitration by RNS was proven on the basis of isotope labeling with an $^{15}N/^{18}O$-enriched nitrate.[42,43]

It seems that tyrosine is the parent compound for most of the nitro aromates described here. Starting with tyrosine, first a mono- and dinitration by RNS occur in the benzene ring. The following formation of nitrated phenylpropionic acids (for example, **5**), tyramines, *N*-acetyltyramines (such as **9**), phenyllactic acids and tyrosols (**8**) is easily explained by reduction, oxidative deamination, decarboxylation and acetylation, and a combination thereof. A similar mechanism has been found for the enzymatic degradation of stephanosporin.[8]

Even the chloro derivatives **16** and **17**, as well as the respective hydroxy derivatives **6** and **8**/**15**, may be produced in this way: It has been shown that diazonium ions can be formed under physiological conditions by the reaction of amines with RNS,[44] and more than 30 stable diazonium salts have been reported as natural products.[7,45] The formation of chloro derivatives may be explained by intermediates **28**/**29**, which could react with water or with chloride in the sense of a Sandmeyer reaction (Figure 1). Furthermore, the formation of **27** could be explained by the reaction of tryptamine through the respective diazonium ion with methanol. Further investigations with isotope labeling to confirm these assumptions are, however, presently not realistic because of the very low yield.

EXPERIMENTAL SECTION

Materials and methods

Nuclear magnetic resonance spectra were measured on Varian Unity 300 (300.145 MHz) and Varian Inova 600 (599.740 MHz) spectrometers with tetramethylsilane as internal standard (Varian Dentschland GmbH, Darmstadt, Germany). ESI mass spectra were recorded on a Quattro Triple Quadrupole Mass Spectrometer, Finnegan TSQ 7000 (Thermo Scientific, Dreieich, Germany) with nano-ESI-API-ion source. ESI-HRMS was measured on a 7 Tesla-Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometer (APEX IV, Bruker Daltonik GmbH, Bremen, Germany). IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer as KBr pellets. UV-VIS spectra were recorded on a Perkin-Elmer Lambda 15 UV/vis spectrometer (Wallthem, MA, USA). Flash chromatography was carried out on silica gel (230–400 mesh). TLC and determination of R_f values were performed on Polygram SIL G/UV_{254} (Macherey-Nagel & Co., Düren, Germany). Size exclusion chromatography was carried out on Sephadex LH-20 (Lipophilic Sephadex, Amersham Bioscience Ltd; purchased from Sigma-Aldrich Chemie, Steinheim, Germany).

HPLC Two Jasco Intelligent Prep. pumps PU-987 (Jasco, Easton, MD, USA) with high-pressure mixer, Vertex 4×250 mm column with 4×4 mm precolumn, Merck Lichrosorb RP C18 7 µm (Merck, Darmstadt, Germany); and preparative separations on Eurochrom Europrep RP 60–10 C18 60 Å 7–12 µm (Knauer, Berlin, Germany) aceotrope (83.7% acetonitrile 16.3% water, b.p. 78.5 °C) were used.

Taxonomy, fermentation and isolation

Details on the producing strain and its cultivation in Erlenmeyer flasks and in a 20-1 fermenter have been published previously.[1]

Isolation and characterization of metabolites

The crude extract from a 20 l batch fermentation was separated on silica gel with a stepwise CH_2Cl_2/MeOH gradient as described before;[1] the fractions obtained were further separated by chromatography on Sephadex LH-20 (CH_2Cl_2/MeOH 2:1 und MeOH). Subsequent flash chromatography on silica gel (CH_2Cl_2/MeOH) afforded 0.4 mg *p*-nitrophenol (**1**), 1.8 mg 4′-hydroxy-3′-nitrobenzoic acid (**2**), 3.0 mg 4′-hydroxy-3′-nitrophenylacetic acid (**3**), 1.7 mg 4′-hydroxy-3′-nitrophenylpropionic acid (**5**), 1.7 mg 4′-hydroxy-3′-nitrophenylacetic acid methyl ester (**4**), 2.6 mg 3′-nitrotyrosol (**8**), 7.1 mg *N*-acetyl-3′-nitrotyramine (**9**), 0.4 mg 2-nitro-4-(2′-nitroethenyl)phenol (**10**), 0.4 mg 4′-hydroxy-3′,5′-dinitrophenylacetic acid methyl ester (**12**), 1.1 mg 4′-hydroxy-3′,5′-dinitrophenylpropionic acid (**13**), 0.6 mg 4′-hydroxy-3′,5′-dinitrophenylethyl chloride (**16**), 0.7 mg 4′-hydroxy-3′,5′-dinitrophenyl-2-chloropropionic acid methyl ester (**17**), 2.7 mg *N*-(4′-hydroxy-3′,5′-dinitrophenylethyl)-acetamide (**18**), 0.4 mg 2,6-dinitro-4-(2′-nitroethenyl)phenol (**19**), 6.1 mg pyriculamide (**25**), 1.1 mg 3′-nitro-daidzein (**22**), 3.9 mg 3′,5′-dinitrogenistein (**24**), 1.2 mg 2-methoxy-4,6-dinitrophenol (**20**), 1.8 mg 3-methoxy-4,5-dinitrophenol (**21**), 2.1 mg 2,6-dimethoxy-1,4-benzoquinone (**26**), 1.1 mg 3′-indolylethyl-methylether (**27**), 4.7 mg thymine, 4.2 mg uracil and 2.2 mg *p*-hydroxybenzoic acid.

The crude extract (1.5 g) obtained from a second 20 l fermentation was separated on silica gel with ethyl acetate/hexane and then with Sephadex LH-20 (MeOH), followed by preparative HPLC (MeCN+0.001% H_3PO_4). The following compounds were obtained: 4′-hydroxy-3′-nitrophenyl-propionic acid (**5**, 1.2 mg), 2-hydroxy-3-(4′-hydroxy-3′-nitrophenyl)propionic acid methyl ester (**6**, 2 mg), 2-chloro-3-(4′-hydroxy-3′-nitrophenyl)propionic acid methyl ester (**7**, 2.1 mg), 4′-hydroxy-3′,5′-dinitrophenylacetic acid (**11**, 2.0 mg), 3-(4′-hydroxy-3′,5′-dinitrophenyl)propionic acid methyl ester (**14**, 0.7 mg), dinitrotyrosol (**15**, 2.7 mg), and 3′-mononitrogenistein (**23**, 1.4 mg). The retention

time of an individual compound corresponds to the elution profile, shown in Supplementary Figure 3.

4′-Hydroxy-3′-nitrobenzoic acid (2)

Yellow solid, UV absorbing (254 nm), R_F 0.25 (CH_2Cl_2/MeOH 9:1), R_t 11.9 min (LC-MS). UV/vis (MeOH) λ_{max} (lg ε) 237 (4.38), 340 (3.51) nm. IR (KBr) ν_{max} 3307, 2924, 2853, 1685, 1626, 1574, 1541, 1433, 1338, 1312, 1257, 1175, 1112, 923, 854, 827, 762, 704, 639, 532 cm^{-1}. ^1H NMR (CD_3OD, 600 MHz) δ 8.66 (d, ^4J 1.8 Hz, 1H, 2-H), 8.15 (dd, ^4J 1.8 Hz, ^3J 8.4 Hz, 1H, 6-H), 7.13 (d, ^3J 8.4 Hz, 1H, 5′-H). ^{13}C NMR (CD_3OD, 150 MHz) δ 169.8 (COOH), 158.0 (C_q-4), 137.9 (CH-6), 135.5 (C_q-3), 127.8 (CH-2), 126.9 (C_q-1), 120.8 (CH-5). EI-MS (70 eV) *m/z* (%) 183 ($[M]^{\bullet+}$, 100), 167 (12), 166 (18), 153 (16), 119 (22), 81 (23), 63 (61), 53 (28). (−)-APCI MS *m/z* 182 [M–H]$^-$.

4′-Hydroxy-3′-nitrophenylacetic acid (3)

Yellow solid, UV absorbing (254 nm), R_F 0.39 (CH_2Cl_2/MeOH 9:1), R_t 11.3 min (LC-MS). UV/vis (MeOH) λ_{max} (lg ε) 274 (3.84), 354 (3.52) nm. IR (KBr) ν_{max} 3432, 2930, 1698, 1632, 1581, 1493, 1434, 1412, 1331, 1258, 1216, 1180, 1132, 1084, 922, 848, 764 cm^{-1}. ^1H NMR (CD_3OD, 300 MHz) δ 8.01 (d, ^4J 2.1 Hz, 1H, 2-H), 7.54 (dd, ^3J 8.5 Hz, ^4J 2.1 Hz, 1H, 6-H), 7.10 (d, ^3J 8.5 Hz, 1H, 5′-H), 3.63 (s, 2H, CH_2COO). EI MS (70 eV) *m/z* (%) 198 (38), 197 ($[M]^{\bullet+}$, 23), 152 (100), 106 (20), 77 (19), 51 (18).

4′-Hydroxy-3′-nitrophenylacetic acid methyl ester (4)

Yellow solid, UV absorbing (254 nm), R_F 0.76 (CH_2Cl_2/MeOH 9:1), R_t 13.6 min (LC-MS). UV/vis (MeOH) λ_{max} (lg ε) 273 (4.00), 354 (3.68) nm. IR (KBr) ν_{max} 3480, 3280, 1736, 1634, 1581, 1537, 1486, 1430, 1420, 1356, 1330, 1307, 1262, 1172, 1083, 1002, 918, 926 cm^{-1}. – ^1H NMR (CD_3OD, 300 MHz) δ 7.99 (d, ^4J 2.4 Hz, 1H, 2-H), 7.52 (dd, ^3J 8.7 Hz, ^4J 2.4 Hz, 1H, 6-H), 7.09 (d, ^3J 8.7 Hz, 1H, 5′-H), 3.69 (s, 3H, COOMe), 3.67 (s, 2H, CH_2). EI MS (70 eV) *m/z* (%) 211 ($[M]^{\bullet+}$, 16), 165 (11), 152 (100), 135 (12), 106 (44), 77 (36), 59 (23), 51 (35).

4′-Hydroxy-3′-nitrophenylpropionic acid (5)

Yellow solid, UV absorbing (254 nm), R_F 0.82 (CH_2Cl_2/MeOH 9:1), R_t 11.4 min (LC-MS). UV/vis (MeOH) λ_{max} (lg ε) 274 (3.65), 356 (3.31) nm. IR (KBr) ν_{max} 3404, 2922, 1714, 1630, 1581, 1539, 1483, 1430, 1404, 1327, 1244. 1180, 1081, 907, 850, 762, 661, 600 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 10.46 (s, 1H, 4′-OH), 7.93 (d, ^4J 2.1 Hz, 1H, 2-H), 7.43 (dd, ^3J 8.4 Hz, ^4J 2.1 Hz, 1H, 6-H), 7.08 (d, ^3J 8.4 Hz, 1H, 5′-H), 2.93 (t, ^3J 7.0 Hz, 2H, CH_2CH_2COO), 2.67 (t, ^3J 7.0 Hz, 2H, CH_2CH_2COO); (MeOH-d_4, 600 MHz) δ 7.96 (d, *J* 2.1 Hz, 1H, 2-H), 7.52 (dd, *J* 8.4, 2.1 Hz, 1H, 6-H), 7.08 (d, *J* 8.4 Hz, 1H, 5′-H), 2.90 (t, *J* 7.0 Hz, 2H, CH_2CH_2COO), 2.59 (t, *J* 7.0 Hz, 2H, CH_2CH_2COO). EI MS (70 eV) *m/z* (%) 211 ($[M]^{\bullet+}$, 32), 193 (46), 175 (20), 152 (100), 151 (56), 147 (38), 106 (15).

2-Hydroxy-3-(4′-hydroxy-3′-nitrophenyl)-propionic acid methyl ester (6)

Yellow solid, R_F 0.59 (CH_2Cl_2/MeOH 9:1), R_t 9.14 min (LC-MS). UV/vis (MeOH) λ_{max} nm (lg ε) 274 (3.45), 354 (3.07). IR (KBr) ν_{max} 3433, 2925, 2853, 1740, 1630, 1538, 1489, 1431, 1384, 1329, 1253, 1181, 1098, 824, 765, 678 cm^{-1}. ^1H NMR (MeOH-d_4, 600 MHz) δ 7.96 (d, ^4J 2.1 Hz, 1H, 2-H), 7.50 (dd, ^3J 8.4 Hz, ^4J 2.1 Hz, 1H, 6-H), 7.08 (d, ^3J 8.4 Hz, 1H, 5′-H), 4.38 (AB*X*, 1H, 2-H), 3.70 (s, 3H, 1-OCH_3), 3.06, 2.94 (ABX, J_{AB} 13, J_{AX} 8.4, J_{BX} 4.2 Hz, 2H, 3-H_2); see Supplementary Figure 8. EI (70 eV) *m/z* (%) 241 ($[M]^{\bullet+}$, 8), 223 (24), 192 (3), 152 (100), 135 (11), 106 (20), 77 (21). (−)-APCI MS *m/z* 240 [M-H]$^-$. (−)-ESI-HRMS *m/z* 240.05147 ([M-H]$^-$, calcd. 240.05135 for $C_{10}H_{10}NO_6$).

2-Chloro-3-(4′-hydroxy-3′-nitrophenyl)propionic acid methyl ester (7)

Yellow solid, UV absorbing (254 nm), R_t 10.54 min (LC-MS). –UV/vis (MeOH) λ_{max} (lg ε) 270 (3.75), 348 (3.42) nm. IR (KBr) ν_{max} 3423, 2926, 1746, 1631, 1540, 1491, 1436, 1328, 1256, 1177, 824, 765 cm^{-1}. ^1H NMR (MeOH-d_4, 600 MHz) δ 7.96 (d, ^4J 2.1 Hz, 1H, 2-H), 7.50 (dd, ^3J 8.4 Hz, ^4J 2.1 Hz, 1H, 6-H), 7.08 (d, ^3J 8.4 Hz, 1H, 5′-H), 4.63 (t, 1H, ^3J 7 Hz, 2-H), 3.73 (s, 3H, 1-OCH_3), 3.75, 3.10 (2 m, 2H, 3-H_2). – EI (70 eV) *m/z* (%) 259, 261 ($[M]^{\bullet+}$, 6, 2), 223 (100), 192 (50), 152 (87), 106 (19), 77 (17). (−)-APCI MS *m/z* 258, 260 [M-H]$^-$. (−)-ESI-HRMS *m/z* 258.01767 ([M+-H]$^-$, calcd. 258.01747 for $C_{10}H_9ClNO_5$).

3′-Nitrotyrosol (8)

Yellow solid, UV absorbing (254 nm), R_F 0.59 (CH_2Cl_2/MeOH 9:1), R_t 13.2 min (LC-MS). UV/vis (MeOH) λ_{max} (lg ε) 275 (3.93), 358 (3.59) nm. IR (KBr) ν_{max} 3300, 2929, 2847, 1632, 1586, 1540, 1488, 1427, 1326, 1253, 1179, 1058, 1026, 904, 836, 764 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 10.46 (s, 1H, 4′-OH), 7.96 (d, ^4J 2.2 Hz, 1H, 2-H), 7.46 (dd, ^3J 8.5 Hz, ^4J 2.2 Hz, 1H, 6-H), 7.08 (d, ^3J 8.5 Hz, 1H, 5′-H), 3.86 (t, ^3J 6.5 Hz, 2H, CH_2CH_2OH), 2.84 (t, ^3J 6.5 Hz, 2H, CH_2CH_2OH). EI MS (70 eV) *m/z* (%) 183 ($[M]^{\bullet+}$, 35), 152 (100), 135 (39), 106 (31), 77 (20), 51 (9).

N-Acetyl-3′-nitrotyramine (9)

Yellow solid, UV absorbing (254 nm), R_F 0.51 (CH_2Cl_2/MeOH 9:1), R_t 11.1 min (LC-MS). UV/vis (MeOH) λ_{max} (lg ε) 275 (3.86), 357 (3.56) nm. IR (KBr) ν_{max} 3400, 3290, 3079, 2934, 1634, 1558, 1532, 1489, 1424, 1321, 1291, 1257, 1171, 850, 766 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 10.44 (s, H/D exchangeable, 1H, 4′-OH), 7.89 (d, ^4J 2.1 Hz, 1H, 2-H), 7.41 (dd, ^3J 8.4 Hz, ^4J 2.1 Hz, 1H, 5′-H), 7.08 (d, ^3J 8.4 Hz, 1H, 6-H), 5.72 (br s, H/D exchangeable, 1H, NH), 3.46 (q, ^3J 6.9 Hz, 2H, CH_2CH_2NH), 2.79 (t, ^3J 6.9 Hz, 2H, CH_2CH_2NH), 1.93 (s, 3H, Ac). EI MS (70 eV) *m/z* (%) 206 ($[M]^{\bullet+}$, 60), 165 (100), 135 (23), 105 (19), 77 (15), 72 (18), 60 (17), 43 (41).

2-Nitro-4-(2′-nitroethenyl)phenol (10)

Yellow solid, UV absorbing (254 nm), R_F 0.83 (CH_2Cl_2/MeOH 9:1), R_t 13.7 min (LC-MS). UV/vis (MeOH) λ_{max} (lg ε) 222 (4.20), 269 (4.04), 325 (4.20), 435 (sh) (3.54) nm. IR (KBr) ν_{max} 3436, 3110, 2927, 1640, 1624, 1537, 1513, 1504, 1489, 1342, 1275, 1174, 972, 836, 766 cm^{-1}. ^1H NMR (CD_3OD, 600 MHz) δ 8.36 (d, ^4J 2.4 Hz, 1H, 2-H), 8.04 (d, ^3J 13.5 Hz, 1H, $CHNO_2$), 7.86 (d, ^3J 13.5 Hz, 1H, $CHCHNO_2$), 7.85 (dd, ^4J 2.4 Hz, ^3J 8.7 Hz, 1H, 6-H), 7.09 (d, ^3J 8.7 Hz, 1H, 5′-H). ^{13}C NMR (CD_3OD, 150 MHz) δ 160.8 (C_q-4), 145.8 (C_q-3), 138.4 ($CHCHNO_2$), 138.0 ($CHCHNO_2$), 136.5 (CH-6), 129.0 (CH-2), 122.5 (CH-5), 121.8 (C_q-1); see Supplementary Figure 9. EI MS (70 eV) *m/z* (%) 210 ($[M]^{\bullet+}$, 24), 163 (100), 118 (38), 89 (70), 63 (58).

4′-Hydroxy-3′,5′-dinitrophenylacetic acid (11)

Yellow solid, R_F 0.55 (CH_2Cl_2/MeOH 9:1), R_t 12.8 min (LC-MS). UV/vis (MeOH/HCl) λ_{max} (lg ε) 338, (3.97), 350 (3.65) nm. IR (KBr) ν_{max} 3433, 1696, 1641, 1580, 1544, 1431, 1401, 1352, 1305, 1261, 1155, 910, 729, 609 cm^{-1}. ^1H NMR (MeOH-d_4, 600 MHz) δ 8.22 (s, 2H, 2′,6′-H), 3.72 (s, 2H, 2-CH_2). EI-MS (70 eV) *m/z* (%) 242 ($[M]^{\bullet+}$, 65), 197 (100), 151 (20), 105 (16), 76 (16). (−)-APCI *m/z* 241 [M-H]$^-$. (−)-ESI HRMS *m/z* 241.01027 ([M-H]$^-$, calcd. 241.00997 for $C_8H_5N_2O_7$).

4′-Hydroxy-3′,5′-dinitrophenylacetic acid methyl ester (12)

Yellow solid, UV absorbing (254 nm), R_F 0.36 (CH_2Cl_2/MeOH 9:1), R_t 12.8 min (LC-MS). UV/vis (MeOH) λ_{max} (lg ε) 228 (4.33), 432 (3.85) nm. IR (KBr) ν_{max} 3437, 2925, 1738, 1631, 1553, 1439, 1406, 1384, 1338, 1265, 1238, 1198, 1177, 1116, 900, 784 cm^{-1}. ^1H NMR (CD_3OD, 600 MHz) δ 7.96 (s, 2H, 2,6-H), 3.70 (s, 3H, $COOCH_3$), 3.60 (s, 2H, CH_2COO). ^{13}C NMR (CD_3OD, 150 MHz) δ 173.7 (COO), 159.4 (C_q-4), 143.9 (C_q-3,5), 132.4 (CH-1), 114.6 (CH-2,6), 52.6 (CH_2COO), 39.6 ($COOCH_3$). EI MS (70 eV) *m/z* (%) 256 ($[M]^{\bullet+}$, 14), 226 (13), 197 (12), 59 (16), 44 (100). EI HRMS *m/z* 256.03330 ($[M]^{\bullet+}$, calcd. 256.03315 for $C_9H_8N_2O_7$); (+)-ESI-HRMS *m/z* 279.02252 ($[M+Na]^+$, calcd. 279.02238 for $C_9H_8N_2O_7Na$).

4′-Hydroxy-3′,5′-dinitrophenylpropionic acid (13)

Yellow solid, UV absorbing (254 nm), R_f 0.16 (CH_2Cl_2/MeOH 9:1), R_t 11.9 min (LC-MS). UV/vis (MeOH) λ_{max} (lg ε) 356 (3.16), 436 (3.39) nm. IR (KBr) ν_{max} 3412, 1712, 1638, 1542, 1428, 1343, 1262 cm^{-1}. ^1H NMR (CD_3OD, 600 MHz) δ 8.04 (s, 2H, 2,6-H), 2.89 (t, ^3J 7.0 Hz, 2H, CH_2CH_2COO), 2.62 (t, ^3J 7.0 Hz, 2H, CH_2CH_2COO). ^{13}C NMR (CD_3OD, 150 MHz) δ 176.2 (COOH), 152.7 (C_q-4), 141.7 (C_q-3,5), 131.4 (CH-2,6), 128.4 (C_q-1), 36.1 (CH_2CH_2COO), 30.3

(CH_2CH_2COO). (−)-ESI MS *m/z* (%) 533 [2M-2H+Na]$^-$ (29), 255 [M-H]$^-$ (100). EI-HRMS *m/z* 256.03320 ([M]$^{\bullet+}$, calcd. 256.03261 for $C_9H_8N_2O_7$).

3-(4′-Hydroxy-3′,5′-dinitrophenyl)-propionic acid methyl ester (14)

Yellow solid, R_F 0.62 (CH_2Cl_2/MeOH 9:1). EI MS (70 eV) *m/z* (%) 270 ([M]$^{\bullet+}$, 20), 260 (45), 210 (100), 187 (10), 180 (10). (−)-ESI HRMS *m/z* 269.04160 ([M-H]$^-$, calcd. 269.04097 for $C_{10}H_9N_2O_7$).

Dinitrotyrosol (15)

Yellow solid, R_F 0.55 (CH_2Cl_2/MeOH 9:1). R_t 10.34 min (LC-MS). UV/vis (MeOH/HCl) λ_{max} (lg ε) 248 sh (3.80), 351 (3.51) nm. IR (KBr) ν_{max} 3395, 2945, 2834, 1639, 1545, 1384, 1029, 618 cm^{-1}. ^{1}H NMR (CD_3OD, 600 MHz) δ 8.14 (s, 2H, 2′,6′-H), 3.78 (t, 2H, ^3J 6.8 Hz, 2-H_2), 2.82 (t, ^3J 6.8 Hz, 2H, 2-H_2). EI MS (70 eV) *m/z* (%) 228 ([M]$^{\bullet+}$, 32), 197 (28), 180 (100), 151 (28), 105 (12). (−)-APCI MS *m/z* 227 [M-H]$^-$. (−)-ESI HRMS *m/z* 227.03102 ([M-H]$^-$, calcd. 227.03095 for $C_8H_7N_2O_6$).

4′-Hydroxy-3′,5′-dinitrophenylethylchloride (16)

Yellow solid, UV absorbing (254 nm), R_F 0.44 (CH_2Cl_2/MeOH 9:1), R_t 15.6 min (LC-MS). UV/vis (MeOH) λ_{max} (lg ε) 231 (4.16), 435 (3.68) nm; λ_{max} 350 nm in acidic solution. IR (KBr) ν_{max} 3407, 2926, 1719, 1638, 1543, 1459, 1338, 1246, 1111, 10455, 914, 783 cm^{-1}. ^{1}H NMR (CD_3OD, 600 MHz) δ 7.86 (s, 2H, 2,6-H), 3.70 (t, ^3J 7.2 Hz, 2H, CH_2CH_2Cl), 2.93 (t, ^3J 7.2 Hz, 2H, CH_2CH_2Cl). ^{13}C NMR (CD_3OD, 150 MHz) δ 159.6 (C_q-4), 144.2 (C_q-3,5), 132.2 (CH-2,6), 119.1 (C_q-1), 46.0 (CH_2CH_2Cl), 38.2 (CH_2CH_2Cl). EI MS (70 eV) *m/z* (%) 246 ([M]$^{\bullet+}$, 16), 197 (100), 151 (16), 91 (98). EI HRMS *m/z* 246.00430 ([M]$^{\bullet+}$, calcd. 246.00381 for $C_8H_7N_2O_5Cl$).

(4′-Hydroxy-3′,5′-dinitrophenyl)-2-chloropropionic acid methyl ester (17)

Orange solid, UV absorbing (254 nm), R_F 0.41 (CH_2Cl_2/MeOH 9:1), R_t 17.0 min (LC-MS). UV/vis (MeOH) λ_{max} (lg ε) 348 (2.89) nm. IR (KBr) ν_{max} 3427, 2925, 2854, 1740, 1621, 1545, 1399, 1258, 1098, 701 cm^{-1}. ^{1}H NMR (CD_3OD, 600 MHz) δ 7.82 (s, 2H, 2′,6′-H), 4.58 (t, 1H, ^3J 8.9 Hz, 2-H), 3.73 (s, 3H, 1-OCH_3), 3.18, 3.06 (ABX, ^2J 15.1 Hz, J_{AX}, J_{BX} 8.9 Hz, 3-H_2). (−)-APCI MS *m/z* 303 [M-H]$^-$. (−)-ESI-HRMS *m/z* 303.00254 ([M-H]$^-$, calcd. 303.00254 for $C_{10}H_8ClN_2O_7$).

N-Acetyl-3′,5′-dinitrotyramine (18)

Yellow solid, UV absorbing (254 nm), R_F 0.19 (CH_2Cl_2/MeOH 9:1), R_t 10.3 min (LC-MS). UV/vis (MeOH) λ_{max} (lg ε) 225 (3.80), 443 (3.21) nm. IR (KBr) ν_{max} 3421, 2928, 1637, 1543, 1460, 1384, 1362, 1338, 1310, 1253, 1205, 1027, 1002, 910, 785 cm^{-1}. – ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.82 (t br, 1H, NH), 7.64 (s, 2H, 2,6-H), 3.17 (m, 2H, CH_2CH_2NH), 2.48 (m, 2H, CH_2CH_2NH), 1.77 (s, 3H, Ac). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 169.0 (CH_3CO), 158.5 (C_q-4), 142.6 (C_q-3,5), 130.7 (CH-2,6), 114.4 (C_q-1), 40.0 (CH_2CH_2NH), 33.2 (CH_2CH_2NH), 22.5 (CH_3CO). (−)-ESI-MS *m/z* (%) 537 [2M-H]$^-$ (45), 268 [M-H]$^-$ (100). EI HRMS *m/z* 269.06480 ([M], calcd. 269.06425 for $C_{10}H_{11}N_3O_6$); (+)-ESI HRMS *m/z* 270.07206 ([M+H]$^+$, calcd. 270.07206 for $C_{10}H_{12}N_3O_6$).

2,6-Dinitro-4-(2′-nitroethenyl)phenol (19)

Orange solid, UV absorbing (254 nm), R_F 0.53 (CH_2Cl_2/MeOH 9:1). ^{1}H NMR (CD_3OD, 600 MHz) δ 8.24 (s, 2H, 3-H, 5′-H), 7.99 (d, ^3J 13.5 Hz, 1H, 2′-H), 7.76 (d, ^3J 13.5 Hz, 1H, 1′-H). (−)-ESI MS *m/z* 531 [2M-2H+Na]$^-$, 254 [M-H]$^-$; (−)-ESI HRMS *m/z* 254.00545 ([M-H]$^-$, calcd. 254.00546 for $C_8H_4N_3O_7$).

2-Methoxy-4,6-dinitrophenol (20)

Orange solid, UV absorbing (254 nm), R_F 0.50 (CH_2Cl_2/MeOH 9:1), R_t 14.1 min (LC-MS). UV/vis (MeOH) λ_{max} (lg ε) 268 (3.78), 377 (3.89), 420 sh (3.75) nm. IR (KBr) ν_{max} 3440, 1608, 1554, 1493, 1385, 1345, 1235, 1094, 1049, 798, 785, 735, 711 cm^{-1}. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.51 (d, ^4J 2.7 Hz, 1H, 5-H), 7.75 (d, ^4J 2.7 Hz, 1H, 3-H), 3.94 (s, 3H, 2-OCH_3). ^{13}C NMR (CD_3OD, 150 MHz) δ 157.8 (C_q-1), 153.7 (C_q-2), 135.5 (C_q-6), 134.2 (C_q-4), 116.4 (CH-5), 108.3 (CH-3), 57.1 (2-OCH_3). EI MS (70 eV) *m/z* (%) 214 ([M]$^{\bullet+}$, 100), 197 (70), 166 (22), 121 (28), 53 (29), 50 (33).

Compound **20** by synthesis according to Ehrlich and Bogert[14]: ^{1}H NMR (CD_3OD, 300 MHz) δ 8.42 (d, ^4J 2.8 Hz, 1H, 5-H), 7.97 (d, ^4J 2.8 Hz, 1H, 3-H), 4.03 (s, 3H, 2-OCH_3); (acetone-d_6) δ 8.53 (d, ^4J 2.8 Hz, 1H, 5-H), 7.73 (d, ^4J 2.8 Hz, 1H, 3-H), 4.02 (s, 3H, 2-OCH_3); ^{13}C NMR (CD_3OD, 75 MHz) δ 151.4 (C_q-1), 150.3 (C_q-2), 139.9 (C_q-6), 136.4 (C_q-4), 114.0 (CH-5), 110.4 (CH-3), 57.7 (2-OCH_3); ($CDCl_3$) δ 150.8 (C_q-1), 150.5 (C_q-2), 139.2 (C_q-6), 132.4 (C_q-4), 112.6 (CH-5), 111.0 (CH-3), 57.0 (2-OCH_3); (acetone-d_6) δ 157.4 (C_q-1), 153.0 (C_q-2), 139.2 (C_q-6), 132.4 (C_q-4), 112.6 (CH-5), 111.0 (CH-3), 57.3 (2-OCH_3).

3-Methoxy-4,5-dinitrophenol (21)

Orange solid, UV absorbing (254 nm), R_F 0.51 (CH_2Cl_2/MeOH 9:1), R_t 14.1 min (LC-MS). UV/vis (MeOH) λ_{max} (lg ε) 213 (4.22), 266 (3.92), 342 (3.68) nm. IR (KBr) ν_{max} 3391, 3109, 2946, 2835, 1778, 1607, 1556, 1449, 1344, 1261, 1092, 1027, 946, 918, 804, 711 cm^{-1}. ^{1}H NMR (CD_3OD, 600 MHz) δ 8.59 (d, ^4J 2.4 Hz, 1H, 6-H), 7.55 (d, ^4J 2.4 Hz, 1H, 2-H), 3.87 (s, 3H, 3-OCH_3). ^{13}C NMR (CD_3OD, 150 MHz) δ 164.6 (C_q-1), 155.6 (C_q-3), 135.2 (C_q-5), 132.2 (C_q-4), 118.3 (CH-6), 106.7 (CH-2), 57.0 (3-OCH3). (−)-APCI MS *m/z* 213 [M-H]$^-$. (−)-ESI HRMS *m/z* 213.01535 ([M-H]$^-$, calcd. 213.01529 for $C_7H_5N_2O_6$).

2-Methoxy-3,5-dinitrophenol

Nitration of guaiacol acetate (2 g) according to Hynning *et al.*[15] resulted in a mixture of 3- and 5-nitroguaiacol acetate of similar polarity. After drying, the orange resin was dissolved at 0 °C in 5 ml fuming nitric acid and the product precipitated after 5 min by the addition of water. The dinitro acetate was hydrolyzed by dissolving in 2N NaOH. After 5 min at room temperature, the orange-red solution was acidified with diluted HCl and extracted with ether. Some polar impurities were separated by filtration over silica gel (column 4×10 cm, CH_2Cl_2). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.08 (d, ^4J 2.7 Hz, 1H, 4-H), 7.88 (d, ^4J 2.7 Hz, 1H, 6-H), 4.02 (s, 3H, 2-OCH_3). ^{13}C NMR (CD_3OD, 75 MHz) δ 153.7 (C_q-1), 147.6 (C_q-2), 145.2 (C_q-3), 143.7 (C_q-5), 115.3 (CH-6), 111.3 (CH-4), 62.4 (2-OCH_3); assignment on the basis of 2D spectra.

2-Methoxy-4,5-dinitrophenol

2-Methoxy-4,5-dinitrophenol was obtained by nitration of veratrole and ether cleavage, according to Ehrlich and Bogert[14]. –^{1}H NMR (CD_3OD, 300 MHz) δ 7.80 (s, 2H, 3, 6-H), 6.96 (s, 3H, 2-OCH_3). –^{13}C NMR (CD_3OD, 125 MHz) δ 152.8 (C_q-1), 149.3 (C_q-2), 141.9 (C_q-5), 132.9 (C_q-4), 107.7 (CH-3,6), 57.6 (2-OCH_3).

3′-Nitro-daidzein (22)

Yellow solid, UV absorbing 254 nm). R_F 0.59 (CH_2Cl_2/MeOH 9:1). R_t 16.0 min (LC-MS). UV/vis (MeOH) λ_{max} (lg ε) 243 (3.99), 300 (sh, 3.67), 359 (3.22) nm. IR (KBr) ν_{max} 3429, 3281, 1620, 1588, 1578, 1537, 1426, 1385, 1310, 1266, 1240, 1179, 1101, 864, 766 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.90 (s br, 1H, OH), 8.45 (s, 1H, 2-H), 8.15 (d, ^4J 2.1 Hz, 1H, 2′-H), 7.97 (d, ^3J 8.8 Hz, 1H, 5′-H), 7.71 (dd, ^3J 8.8 Hz, ^4J 2.1 Hz, 1H, 6′-H), 7.12 (d, ^3J 8.8 Hz, 1H, 5′-H), 6.94 (dd, ^3J 8.8 Hz, ^4J 2.1 Hz, 1H, 6-H), 6.88 (d, ^4J 2.1 Hz, 1H, 8-H). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 174.3 (C_q-4), 162.8 (C_q-7), 157.4 (C_q-8a), 153.7 (CH-2), 153.0 (C_q-4′), 136.5 (C_q-3′), 135.3 (CH-6′), 127.2 (CH-5), 125.2 (CH-2′), 122.0 (C_q-1′), 121.4 (C_q-3), 119.6 (CH-5′), 116.4 (C_q-4a), 115.3 (CH-6), 102.1 (CH-8). (+)-ESI MS *m/z* (%) 621 [2M+Na]$^+$ (78), 300 [M+H]$^+$ (18). (−)-ESI MS *m/z* (%) 597 [2M-H]$^-$ (40), 298 [M-H]$^-$ (100). DCI MS (NH_3) *m/z* (%) 456 [2M+NH_4]$^+$ (14), 237 [M+NH_4]$^+$ (100). (+)-ESI HRMS *m/z* 300.05020 ([M+H]$^+$, calcd. 300.05027 for $C_{15}H_{10}NO_6$).

3′-Nitrogenistein (23)

Yellow solid, R_F 0.55 (CH_2Cl_2/MeOH 9:1), R_t 12.49 min (LC-MS). UV/vis (MeOH) λ_{max} (lg ε) 265 nm. IR (KBr) ν_{max} 3432, 2963, 2926, 1628, 1537, 1382, 1261, 1092, 1030, 803 cm^{-1}. ^{1}H NMR (MeOH-d_4, 600 MHz) δ 8.20 (s, 1H, 2-H), 8.15 (d, *J* 2.1 Hz, 1H, 6′-H), 7.60 (dd, *J* 8.8, 1.9 Hz, 1H, 2′-H), 7.01 (d, *J* 8.8 Hz, 1H, 3′-H), 6.28 (d, *J* 2.1 Hz, 1H, 8-H), 6.18 (d, *J* 2.1 Hz, 1H, 6-H); see Supplementary Figure 10. (−)-ESI MS *m/z* (%) 314.3 [M-H]$^-$ (100). (−)-ESI

MS/MS (45 eV) *m/z* (%) 314 [M-H]$^-$ (45), 297 (100). (−)-ESI MS/MS (35 eV) *m/z* (%) 297 (100), 280 (90), 267 (80). (+)-APCI MS *m/z* 316 [M+H]$^+$. (−)-APCI MS *m/z* 314 [M-H]$^-$.

3′,5′-Dinitro-genistein (24)

Orange solid, UV absorbing (254 nm), R_F 0.21 (CH_2Cl_2/MeOH 9:1), R_t 12.46 min (LC-MS). –UV/vis (MeOH) λ_{max} (lg ε) 268 (4.12), 434 (3.29) nm. IR (KBr) ν_{max} 3410, 1632, 1552, 1503, 1346, 1258, 1108, 1028, 1006, 822, 557, 450 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.78 (br, 1H, OH), 8.27 (s, 1H, 2-H), 8.03 (s, 2H, 2′,6′-H), 6.15 (d, ^4J 2.1 Hz, 1H, 8-H), 6.03 (d, ^4J 2.1 Hz, 1H, 6-H). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 178.9 (C_q-4), 161.6 (C_q-5), 159.0 (C_q-4′), 157.9 (C_q-8a), 152.6 (CH-2), 142.9 (C_q-3′,5′), 130.4 (CH-2′,6′), 119.9 (C_q-1′), 105.7 (C_q-3), 102.3 (C_q-4a), 100.2 (CH-6), 94.3 (CH-8). (−)-APCI MS *m/z* (%) 359 [M-H]$^-$ (100). (+)-ESI HRMS *m/z* 361.03046 ([M+H]$^+$, calcd. 361.03027 for $C_{15}H_9N_2O_9$); (−)-ESI HRMS *m/z* 359.01580 ([M-H]$^-$, calcd. 359.01569 for $C_{15}H_7N_2O_9$).

Pyriculamide (25)

Yellow solid, UV absorbing (254 nm), R_F 0.45 (CH_2Cl_2/MeOH 9:1), R_T 9.5 min (LC-MS). UV/vis (MeOH) λ_{max} 273, 354 nm. IR (KBr) ν_{max} 3439, 3191, 3081, 2930, 2862, 1655, 1623, 1524, 1472, 1445, 1356, 1335, 1280 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.7 (s br, 1H, OH), 8.14 (d, ^3J 4.0 Hz, 1H, NH), 7.70 (d, ^4J 2.3 Hz, 1H, 5′-H), 7.34 (dd, ^3J 8.4 Hz, ^4J 2.3 Hz, 1H, 9-H), 7.08 (d, ^3J 8.4 Hz, 1H, 8-H), 3.94 (m, 1H, 2-H), 3.48 (m, 1H, 2′-H), 3.46 (m, 1H, 3-H_A), 3.27 (m, 1H, 3-H_B), 3.03, 2.96 (ABX, ^2J 13, ^3J 7 Hz, 2H, 5′-H_2), 2.06 (m, 1H, 3′-H_A), 1.83 (m, 1H, 4′-H_A), 1.68 (m, 2H, 3′-H_A, 4′-H_B). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 168.2 (C_q-1), 164.5 (C_q-1′), 151.1 (C_q-7), 136.6 (CH-5), 136.2 (C_q-6), 127.4 (C_q-4), 125.7 (CH-9), 119.1 (CH-8), 57.8 (CH-2), 57.3 (CH-2′), 44.7 (CH_2-3), 37.7 (CH_2-5′), 28.5 (CH_2-3′), 21.4 (CH_2-4′). (+)-ESI MS *m/z* (%) 655 [2M-H+2Na]$^+$ (100), 633 [2M+Na]$^+$ (25). (−)-ESI MS *m/z* (%) 631 [2M-2H+Na]$^-$ (100), 304 [M-H]$^-$ (55).

2,6-Dimethoxy-1,4-benzoquinone (26)

Yellow solid, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.84 (s, 2H, 3′,5′-H), 3.80 (s, 6H, 2 OCH_3). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 186.8 (C_q-1), 176.7 (C_q-4), 157.3 (C_q-2,6), 107.4 (CH-3,5), 56.5 (2 OCH_3). EI MS (70 eV) *m/z* (%) 168 ([M]$^{\bullet+}$, 5), 104 (39), 91 (21), 80 (20), 69 (100), 53 (29).

3′-Indolylethyl-methyl ether (27)

Colorless oil, UV absorbing at 254 nm, R_F 0.80 (CH_2Cl_2/MeOH 9:1), with anisaldehyde/sulfuric acid red, with Ehrlich's reagent violet. R_t 15.7 min (LC-MS). UV/vis (MeOH) λ_{max} 221, 281 nm. IR (KBr) ν_{max} 3415, 2926, 1620, 1457, 1384, 1339, 1228, 1107, 742 cm^{-1}. ^{1}H NMR (CD_3OD, 600 MHz) δ 7.51 (d, ^3J 7.8 Hz, 1H, 7′-H), 7.31 (d, ^3J 7.8 Hz, 1H, 4′-H), 7.06 (t, ^3J 7.8 Hz, 1H, 6′-H), 7.05 (s, 1H, 2′-H), 6.98 (t, ^3J 7.8 Hz, 1H, 5′-H), 3.66 (t, ^3J 7.2 Hz, 2H, 1-H), 3.37 (s, 3H, OCH_3), 2.99 (t, ^3J 7.2 Hz, 2H, 2-H). EI MS (70 eV) *m/z* (%) 175 ([M]$^{\bullet+}$, 20), 130 (100), 103 (7), 77 (10), 45 (12). EI HRMS *m/z* 175.0997 ([M]$^{\bullet+}$, calcd. 175.09917 for $C_{11}H_{13}NO$).

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1 Al-Zereini, W., Schuhmann, I., Laatsch, H., Helmke, E. & Anke, H. New aromatic nitro compounds from *Salegentibacter* sp. T436, an Arctic sea ice bacterium. Taxonomy, fermentation, isolation and biological activities. *J. Antibiot.* **60,** 301–308 (2007).
2 Shao, C. L. *et al.* Five nitro-phenyl compounds from the South China Sea mangrove fungus. *J Asian Nat Prod Res* **9,** 643–648 (2007).
3 Hoefle, G. & Irschik, H. Isolation and biosynthesis of aurachin P and 5-nitroresorcinol from *Stigmatella erecta. J Nat Prod* **71,** 1946–1948 (2008).
4 Schuhmann, I. Aufbau einer HPLC-UV-ESI-MS/MS-Datenbank und ihre Anwendung im Screening arktischer und antarktischer Meeresbakterien. PhD Thesis, University of Göttingen, Germany (2005).
5 Fotso Fondja Yao, C. B. Aqabamycins, Rare Nitro Maleimides and other Novel Metabolites from Microorganisms; Generation and Application of an HPLC-UV-ESI MS/MS Database. PhD Thesis, University of Göttingen, Germany (2008).
6 Al-Zereini, W. Natural Products from Marine Bacteria. PhD Thesis, Technical University Kaiserslautern (2006).
7 Laatsch, H. AntiBase 2008. A Data Base for Rapid Dereplication and Structure Determination of Microbial Natural Products, Wiley-VCH, Weinheim, Germany; see http//wwwuser.gwdg. de/~ucoc/laatsch/AntiBase.htm.
8 Lang, M., Spiteller, P., Hellwig, V. & Steglich, W. Stephanosporin, a 'traceless' precursor of 2-chloro-4-nitrophenol in the gasteromycete *Stephanospora caroticolor. Angew. Chem. Int. Ed.* **40,** 1704–1705 (2001).
9 Sviridov, S. I. & Ermolinskii, B. S. Secondary metabolites of *Pyricularia oryzae.* I. Derivatives of *o*-nitrophenol. *Khim. Prirod. Soed.* 811–18 (1990) *Chem. Nat. Comp.* **26,** 691–696 (1990).
10 Fleifel, A. M. Syntheses of β-aroyl-α- and β-methylacrylic acids. *J. Org. Chem.* **24,** 1343–1345 (1959).
11 Bruice, T. C. A new 'meta' analog of thyroxine. *J. Org. Chem.* **19,** 333–337 (1954).
12 Tomita, K. & Lardy, H. A. Enzymic conversion of iodinated thyronines to iodinated thyroacetic acids. *J. Biol. Chem.* **235,** 3292–3297 (1960).
13 Svensson, M., Helgee, B., Skarp, K., Andersson, G. & Hermann, D. Phase properties in relation to mesogen length in chiral side-chain polysiloxanes. *Polymer* **38,** 3269–3278 (1997).
14 Ehrlich, J. & Bogert, M. T. Experiments in the veratrole and quinoxaline groups. *J. Org. Chem.* **12,** 522–534 (1947).
15 Hynning, P. A., Remberger, M. & Neilson, A. H. Synthesis, gas-liquid chromatographic analysis and gas-chromatographic-mass spectrometric identification of nitrovanillins, chloronitrovanillins, nitroguaiacols and chloronitroguaiacols. *J. Chromatogr.* **467,** 99–110 (1989).
16 Ohta, K. Chemical studies on biologically-active substances in seaweeds. *Proc. Int. Seaweed Symp.* **9,** 401–411 (1979).
17 Ohta, K. & Takagi, M. Antimicrobial compounds of the marine red alga *Marginisporum aberrans. Phytochemistry* **16,** 1085–1086 (1977).
18 Shangguan, D., Jiang, R., Li, B., Xiao, C. & Wu, J. Production, isolation and structure elucidation of novel isoflavonoid compounds K3-D4, K3-D5, K3-D6. *Zhongguo Kangshengsu Zazhi* **24,** 254–257 (1999).
19 Jiang, R., Li, B., Xiao, C., Yang, D. & Wu, J. Production, isolation and structure elucidation of new isoflavonoid compound K3-D3. *Zhongguo Kangshengsu Zazhi* **22,** 81–83 (1997).
20 Cordell, G. A., Chang, P. T. O., Fong, H. H. S. & Farnsworth, N. R. Xylosmacin, a new phenolic glucoside ester from *Xylosma velutina* (Flacourtiaceae). *Lloydia* **40,** 340–343 (1977).
21 Robinson, G. C. Production of Indoles US 3534059. *Chem. Abstr.* **74,** 53516 (1970).
22 Yinon, J. & Laschever, M. Reduction of trinitroaromatic compounds in water by chemical ionization mass spectrometry. *Org. Mass Spectrom.* **16,** 264–266 (1981).
23 Harrison, A. G. & Kallury, R. K. M. R. Chemical ionization mass spectra of mono-nitroarenes. *Org. Mass Spectrom.* **15,** 284–288 (1980).
24 Brophy, J. J., Diakiw, V., Goldsack, R. J., Nelson, D. & Shannon, J. Anomalous ions in the chemical ionization mass spectra of aromatic nitro and nitroso compounds. *Org. Mass Spectrom.* **14,** 201–203 (1979).
25 Karancsi, T. & Slegel, P. Reliable molecular mass determination of aromatic nitro compounds elimination of gas-phase reduction occurring during atmospheric pressure chemical ionization. *J. Mass. Spectrom.* **34,** 975–977 (1999).
26 Gillis, R. G., Lacey, M. J. & Shannon, J. S. Chemical ionisation mass spectra of explosives. *Org. Mass Spectrom.* **9,** 359–364 (1974).
27 Vats, S., Stuttard, C. & Vining, L. C. Transductional analysis of chloramphenicol biosynthesis genes in *Streptomyces venezuelae. J. Bacteriol.* **169,** 3809–3813 (1987).
28 Siddiqueullah, M., McGrath, R. M., Vining, L. C., Sala, F. & Westlake, D. W. S. Biosynthesis of chloramphenicol. II. p-Aminophenylalanine as a precursor of the p-nitrophenylserinol moiety. *Can. J. Biochem.* **45,** 1881–1889 (1967).
29 Cardillo, R. *et al.* Biosynthesis of aureothin. *Tetrahedron* **30,** 459–461 (1974).
30 Kirner, S. & van Pée, K. H. Biosynthesis of nitro compounds: enzymic oxidation of a precursor with an amino group to pyrrolnitrin. *Angew. Chem.* **106,** 346–347 (1994); Angew Chem, Int Ed Engl 33: 351-352 (1994).
31 van Pée, K. H., Salcher, O. & Lingens, F. Formation of pyrrolnitrin and 3-(2-amino-3-chlorophenyl)pyrrole from 7-chlorotryptophan. *Angew. Chem.* **92,** 855–856 (1980); Angew Chem Int Ed Engl 19: 828–829 (1980).
32 Hütter, K. *et al.* Viriplanin A, a new anthracycline antibiotic of the nogalamycin group. I. Isolation, characterization, degradation reactions and biological properties. *J. Antibiot.* **39,** 1193–1204 (1986).
33 Li, Y., Gould, S. J. & Proteau, P. J. Biosynthesis of 3-amino-4-hydroxybenzoic acid in *Streptomyces murayamaensis* incorporation of [4-13C]oxalacetate. *Tetrahedron Lett.* **41,** 5181–5185 (2000).
34 Kurobane, I., Dale, P. L. & Vining, L. C. Characterization of new viridomycins and requirements for production in cultures of *Streptomyces griseus. J. Antibiot.* **40,** 1131–1139 (1987).
35 Martin, J. H., Gordon, R. M. & Fitzwater, S. E. Iron in Antarctic waters. *Nature* **345,** 156–158 (1990).
36 Guan, L. L., Kanoh, K. & Kamino, K. Effect of exogenous siderophores on iron uptake activity of marine bacteria under iron-limited conditions. *Appl. Environ. Microbiol.* **67,** 1710–1717 (2001).

37 Martin, J. H. & Fitzwater, S. E. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. *Nature* **331,** 341–343 (1988).
38 Rysgaard, S. & Glud, R. N. Anaerobic N_2 production in Arctic sea ice. *Limnol. Oceanogr.* **49,** 86–94 (2004).
39 Radi, R. Nitric oxide, oxidants, and protein tyrosine nitration. *Proc. Natl Acad. Sci. USA* **101,** 4003–4008 (2004).
40 Greenacre, S. A. B. & Ischiropoulos, H. Tyrosine nitration: localization, quantification, consequences for protein function and signal transduction. *Free Radical Res.* **34,** 541–581 (2001).
41 Prasain, J. K. *et al.* Mass spectrometric methods for the analysis of chlorinated and nitrated isoflavonoids A novel class of biological metabolites. *J. Mass Spectrom.* **38,** 764–771 (2003).
42 Carter, G. T. *et al.* Direct biochemical nitration in the biosynthesis of dioxapyrrolomycin. A unique mechanism for the introduction of nitro groups in microbial products. *J. Chem. Soc., Chem. Commun.* **17,** 1271–1273 (1989).
43 Ratnayake, A. S. *et al.* Investigating the biosynthetic origin of the nitro group in pyrrolomycins. *J. Nat. Prod.* **71,** 1923–1926 (2008).
44 Backhaus, C., Rahman, H., Scheffler, S., Laatsch, H. & Hardeland, R. NO scavenging by 3-hydroxyanthranilic acid and 3-hydroxykynurenine N-nitrosation leads to oxadiazoles and their tautomers, *o*-quinone diazides. *Nitric Oxide Biol. Chem.* **19,** 227–244 (2008).
45 Dornberger, K. *et al.* Antibiotics from basidiomycetes. Evidence for the occurrence of the 4-hydroxybenzenediazonium ion in the extracts of *Agaricus xanthodermus* Genevier (Agaricales). *Tetrahedron Lett.* **27,** 559–560 (1986).

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