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First total synthesis and phytotoxic activity of *Streptomyces* sp. metabolites abenquines

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ABSTRACT

The first total synthesis of abenquines A, B2, C and D has been achieved in three steps starting from commercially available 2,5-dimethoxyaniline, with overall yields of 41–61%. Four analogues bearing the amino acids D-valine (**17**), L-methionine (**18**), and glycine (**19**), and benzylamine (**20**), were also prepared in 45–72% yield. The inhibitory properties of these compounds were evaluated against the photoautotrophic growth of a model *Synechococcus* sp. strain. Abenquine C and its enantiomer were substantially ineffective, whereas all other abenquines significantly inhibited cell proliferation, with concentrations causing 50%-inhibition of algal growth ranging from 10^{-5} to 10^{-6} M.

Introduction

Among the plethora of natural products, such as polyketides, terpenoids, steroids, alkaloids, amino acids, and carbohydrates,[1] the quinones represent an important class of substances endowed with a vast array of biological activities.[2] These compounds are found in plants, microorganisms, and marine organisms.[2] Despite the large structural variation among the natural quinones, those bearing an amino group *ortho* to the carbonyl group are less common. One of the earliest natural aminoquinones identified is streptonigrin (**1**) (Fig. 1), a highly functionalized antibiotic isolated from *Streptomyces flocculus* in 1959.[3] This compound entered phase II clinical trials as an anticancer agent in the 1970s, and has attracted the attention of many research groups.[4] In the early 1980s lavendamycin (**2**), structurally related to streptonigrin, was isolated from *Streptomyces lavendulae*, and was shown to exert cytotoxic and antimicrobial activities.[5] Lavendamycin was also the object of synthetic investigations, including the preparation of analogues for biological studies.[4b,6]

In 1994 two new terpenoid aminoquinones, nakijiquinones A (**3**) and B (**4**), were isolated from a marine sponge (family Spongiidae). Besides representing the first sesquiterpenoid quinones with amino acid residues of natural origin, they also exhibited cytotoxic activity against some cancer cell lines.[7] This discovery was followed by the isolation[8] and synthesis[9] of several new nakijiquinones and analogues bearing amino acid residues. One aminoquinone structurally related to nakijiquinones was then isolated from the marine sponge *Dactylospongia elegans* and named smenospongine.[10] This compound and other natural analogues isolated from marine sources presented antimicrobial and cytotoxic activities.[11] Other natural aminoquinones endowed with biological activities include metachromins,[12] geldanamycin,[13] mytomicin,[14] and dysifragilones.[15]

Recently, a new group of benzoquinones bearing natural amino acid residues (abenquines A–D, **6**–**10**) (Fig. 1) were isolated from a strain of *Streptomyces* sp. collected from the Atacama desert in Chile. These abenquines showed cytotoxic activity against bacteria and dermatophytic fungi, and were inhibitory of phosphodiesterase type 4b activity.[16] Several groups,[17] including ours,[18] have described aminobenzoquinone phytotoxicity, but little work has been done to address their development as new herbicides. In view of our interest in this area and considering that abenquines are available in short supply, we report here the first total synthesis of such compounds and a preliminary assessment of their phytotoxic effects.

Results and discussion

Initially we envisaged that all abenquines could be obtained from quinone **11**, which in turn could easily be prepared from the commercially available 2,5-dimethoxyaniline **13** (Scheme 1).

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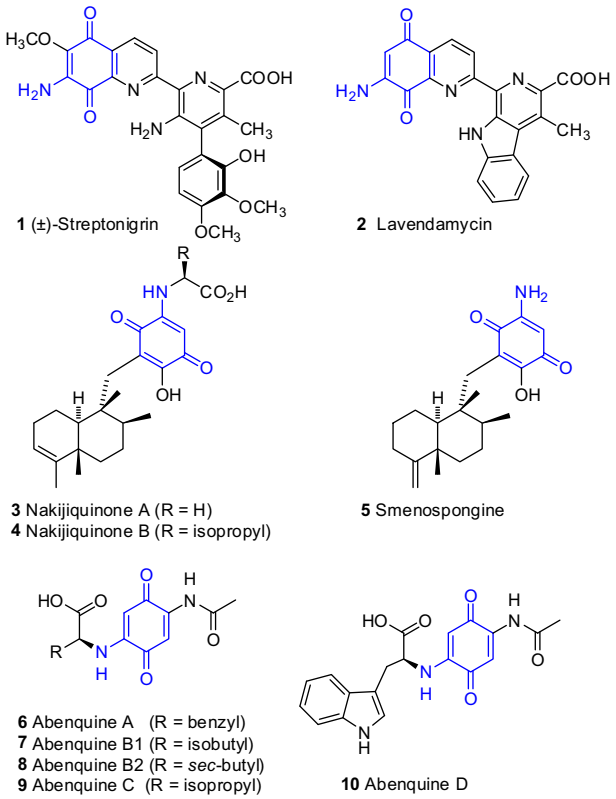
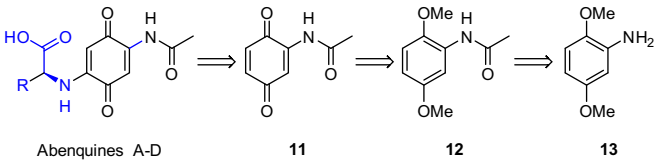


Figure 1. Structures of some natural aminoquinones.

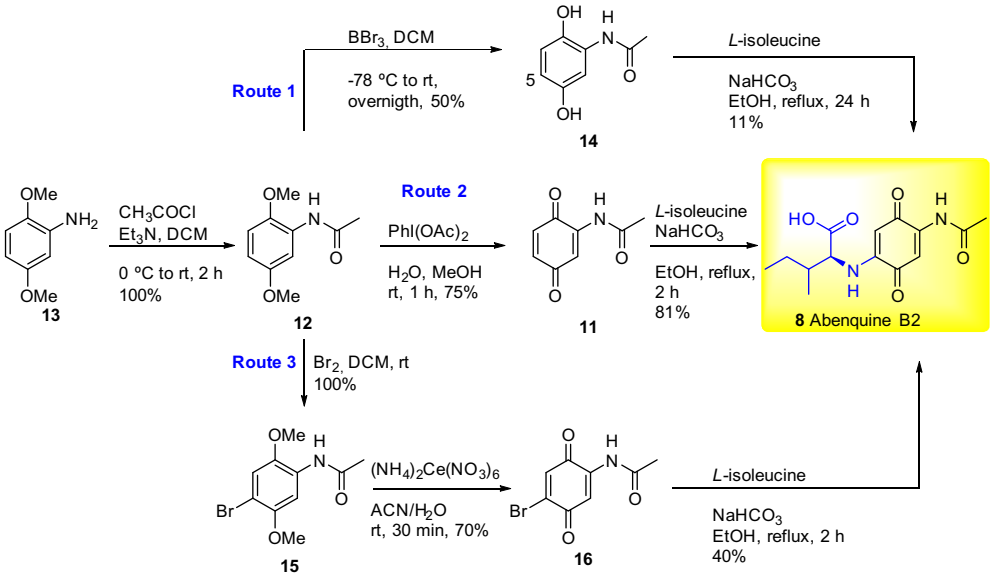


Scheme 1. Retrosynthetic analysis of abenquines A–D.

With this plan in mind, compound **13** was treated with acetyl chloride and quantitatively converted into amide **12** on a 2 g scale (Scheme 2). We then investigated the conversion of this amide into abenquine B2 (**8**) via three routes, as shown in Scheme 2. Initially compound **12** was demethylated by treatment with BBr_3 using a methodology previously applied for the synthesis of alternariol analogues.[19] Although the required hydroquinone **14** was obtained in low yield (50%), no attempt was made to optimize this reaction step since we focused on the investigation of a direct conversion of **14** into abenquine B2 using the methodology proposed by Zhong-Lu et al.[20] Thus, we reacted **14** with isoleucine in ethanol for 24 h, but the required product **8** was isolated in only 11% yield. This low yield was not surprising, since we have recently shown that a direct amination followed by spontaneous oxidation of hydroquinone is a low efficiency process.[18b]

Based on our recent success in obtaining 2,5-bis-(alkyl/arylamino)-1,4-benzoquinones directly from benzoquinones,[18b] we then converted compound **12** into benzoquinone **11**. As reported by Whiteley,[21] the oxidation of **12** with a large excess of cerium ammonium nitrate (CAN) in acetonitrile and water can afford the target product **11**. When we attempted the reaction using only two CAN equivalents, the reaction gave a complex mixture of polar compounds. This result is in agreement with previous data showing that 1,4-dimethoxybenzene with one substituent gives rise to a dimerization reaction.[22] An alternative treatment of **12** with Dess–Martin periodinane (DMP) resulted in the required quinone **11** in a better yield of 50%. To improve it further, a hypervalent iodine reagent, namely phenyliodine diacetate (PIDA),[23] was used and afforded quinone **11** in a 75% yield. Despite repeating this reaction several times, the yield could not be increased anymore, as other side products always formed.

Compound **11** was subsequently reacted with isoleucine in the presence of sodium bicarbonate in ethanol. After 2 h at reflux, the required abenquine B2 was isolated in 81% yield. Therefore, starting from the commercially available aniline **13**, abenquine B2 was obtained in just 3 steps and 60.8% yield. The structure of this compound was proven by extensive spectroscopic analysis. The molecular formula $C_{14}H_{18}N_2O_5$ was established by HRMS (ESI), in accordance with the signal at $m/z = 295.1290$ ($[M+H]^+$). The IR spectra of abenquine B2 showed absorptions typical of amino acid groups (broad band at 3404–2736 cm^{-1}), and another band at



Scheme 2. Investigated routes for the synthesis of abenquine B2.

1626 cm^{-1} due to stretch vibration of the amide carbonyl group.[24] The ^1H NMR spectrum showed a singlet at 2.22 ppm (MeCO group) and another singlet at 5.43 ppm typical of the vinylic H-2′ and the H-2 of amino acid as doublet at 3.99 ppm. The ^{13}C NMR spectrum analysis confirmed the carbonyl group of the amide at 172.7 ppm and the signals at 11.7, 15.6, 26.7, 38.2, 64.4 ppm associated with the amino acid residues. A detailed assignment of the NMR spectra (^1H and ^{13}C) and other physical data for this compound are presented as Supplementary data. Although this compound has been described previously, no spectroscopic data were reported in the literature,[16] and a comparison was not possible.

A further attempt (route 3) to improve the overall yield of this synthesis involved the initial bromination of amide **13** to afford **15** in quantitative yield. The rationale for this transformation was that, according to Hayashi et al.,[22c] the oxidation of a disubstituted 1,4-dimethoxybenzene derivative proceeds in better yield and produces less dimer than compound **13**. We then oxidized compound **15** employing the same reagents used for the production of **11**. It was found that the use of CAN and PIDA afforded the bromoquinone **16** in 70% yield, while the treatment with DMP did not produce the required compound.

Based on the efficient substitution of a bromine for an amino group in case of 2,3-dibromofuran-2(5*H*)-one,[25] we envisaged that the same reaction would take place with compound **16**. Consequently, we reacted **16** with isoleucine under the conditions reported above, but abenquine B2 was formed in a disappointing 40% yield. In summary, the shortest and most efficient approach for the preparation of abenquine B2 in a satisfactory yield consisted of route 2. This procedure afforded abenquines A (**6**), C (**9**) and D (**10**) in 68%, 70% and 55% yield, respectively (Scheme 3).

The structures of abenquines A, C, and D were confirmed by spectroscopic analysis. For all compounds (**6**, **9**, **10**) the infrared spectra presented a strong absorption in the range 3554–2794 cm^{-1} corresponding to the amino acid group. In the ^1H NMR spectra, the methyl group of amide was observed for all compounds as a singlet at 2.21–2.18 ppm. In addition, the vinylic hydrogen-2′ is located at 5.24–5.39 ppm and the hydrogen-2 corresponding to amino acid at 3.70–4.12 ppm. The ^{13}C NMR spectra confirmed the carbonyl group of the amide (signals at 172.6–172.7 ppm) and the carboxylic carbon at 176.4–177.1 ppm. The high resolution mass spectra for all three compounds were in agreement with the corresponding molecular formulas. A detailed assignment of the NMR spectra (^1H and ^{13}C) and physical data for each compound are presented in the Supplementary data (Experimental section). All spectroscopic data were in agreement with previously reported data.[16]

Having produced for the first time four natural abenquines, we applied the developed methodology to the synthesis of analogues in order to further study their biological activities. In view of that, starting from intermediate **11**, an enantiomer of abenquine C (**17**) was prepared in 68% yield. Other abenquine analogues (**18** and **19**) were obtained in 45% and 60% yield. A simple derivative of benzylamine (**20**) was also obtained in 72% yield (Fig. 2).

The phytotoxicity of abenquines and their analogues was assessed as their ability to inhibit the photoautotrophic growth of a model cyanobacterial strain, *Synechococcus elongatus* PCC6301, using a methodology previously described for some 2,5-diaminobenzoquinones.[18b] The addition to the culture medium of increasing levels of a given compound in the range from 1 to 100 μM in all cases determined a significant reduction of the growth rate constant. A mild effect was evident for abenquine C and its isomer **17**, with a concentration inhibiting growth by 50% (IC_{50}) higher than 10^{-4} M (Table 1). For the other

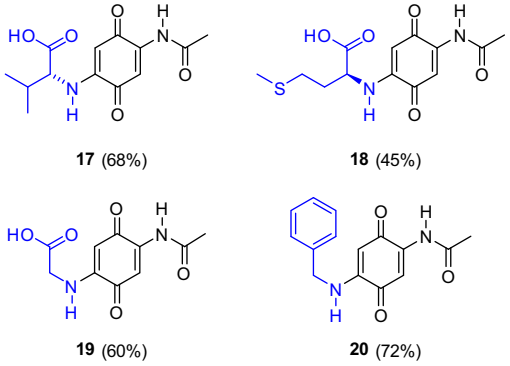
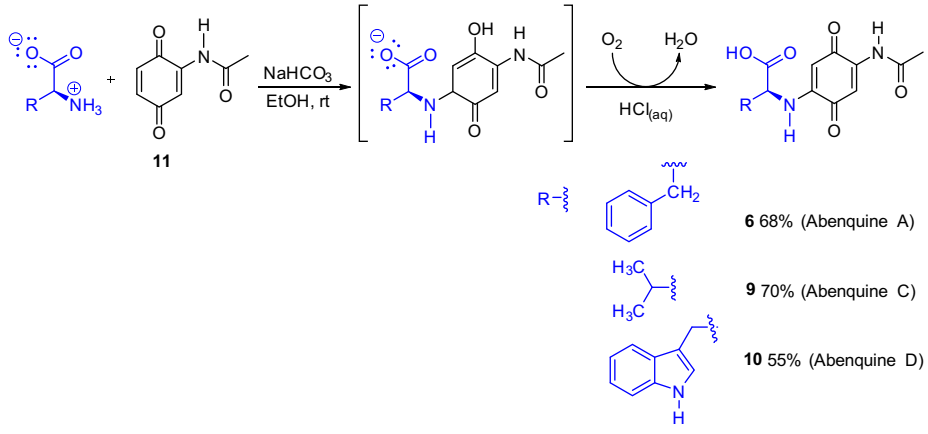


Figure 2. Abenquine analogues prepared.

Table 1
Abenquine concentrations causing 50%-inhibition of cyanobacterial growth

Compound	IC_{50}
6	$9.6 * 10^{-6}$ M
8	$1.4 * 10^{-5}$ M
9	$>10^{-4}$ M
10	$1.2 * 10^{-5}$ M
17	$2.3 * 10^{-4}$ M
18	$5.5 * 10^{-6}$ M
19	$7.7 * 10^{-6}$ M
20	$2.0 * 10^{-6}$ M



Scheme 3. Synthesis of abenquines A, C and D.

compounds the treatment was much more effective, and at the highest doses tested algal growth was completely abolished. Natural abenquines were almost equipotent, with IC_{50} values around 10^{-5} M. The other synthetic analogues were significantly more effective, and in the case of compound **20** an IC_{50} value of $2.0 * 10^{-6}$ M was found. The occurrence of this variability in a limited number of analogues seems very promising for the future tailoring of their scaffold aimed at increasing their effectiveness (Supplementary information).

In conclusion, we have developed a short and efficient method for the synthesis of natural abenquines that can also been applied to the preparation of analogues. These compounds were shown to exert phytotoxic activity against the cyanobacterium *Synechococcus elongatus*. Further work is underway in our laboratories to elucidate their mechanism(s) of action and to produce more active analogues with potential use as herbicides.

Acknowledgments

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Supplementary data

Supplementary data (full experimental procedures and characterization data for all compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.03.038.

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