Studies on the [2+3] cycloaddition reaction of nitrile oxides to abietic acid esters

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Abstract: [2+3] Dipolar cycloadditions of aromatic nitrile oxides to abietic acid esters were investigated. The reactions showed complete site selectivity and regioselectivity, while the stereoselectivity depended on the structures of the dipolarophiles.

Keywords: dipolar cycloaddition; abietates; site selectivity; NMR spectroscopy.

INTRODUCTION

Abietic acid (**1**), as other diterpene resin acids, belongs to the defense system of conifers against potential herbivores and pathogens as an anti-feedant. Abietic acid displays inhibitory activity against chitinase and *β*-glucosidase enzymes in boreal forest soil.[1]

The acid shows antimicrobial, anti-ulcer, cardiovascular, anti-allergic, and surfactant activities,[2] and exhibits anti-inflammatory, phytoalexin-like, and anti-convulsant activities. Abietic acid inhibited both types of 11*β*-hydroxysteroid dehydrogenase, which influences sodium retention and hypertension.[3]

Abietic acid ammonium salts are biodegradable surfactants showing antimicrobial activity.[4] Several C18-oxygenated derivatives were synthesized and evaluated for their cytotoxic, antimycotic, and antiviral activities. However, only the presence of a formyl group improved the bioactivity (both antifungal and anti-tumor one), while carboxy or hydroxy groups were detrimental to the activity.[5] The *in vitro* antioxidant activity of an abietic acid-derived catechol derivative was evaluated.[6]

The [2+3] cycloaddition of nitrile oxides to alkenes is the most convenient and attractive method for the preparation of 2-isoxazolines.[7] These heterocycles are found in a large number of natural products[8] and are valuable intermediates in the synthesis of several pharmaceuticals.[9]

1073

These dipoles can be prepared either by the Huisgen method from the corresponding hydroximinoyl chlorides by base-induced dehydrohalogenation,[7] or by dehydration of primary nitro compounds by phenyl isocyanates (Mukayama method)[10] or ethyl chloroformate (Shimizu method).[11]

The reaction is characterized by a high degree of the *cis*-stereospecificity, *i.e.*, 4,5-*anti* isomers are produced from *E*-alkenes and 4,5-*syn* products are obtained from *Z*-alkenes.[12] Reactions of monosubstituted and 1,1-disubstituted alkenes furnish regioselectively 5-substituted 2-isoxazolines. However, 1,2--disubstituted olefins usually afford mixtures of regio- and stereoisomers.

Cycloadditions of aryl nitrile oxides to alkene-substituted norbornene derivatives were characterized by a high degree of site-selectivity and only the double bond of the norbornene system participated in the reactions.[13,14] On the other hand, in the reaction of polyunsaturated cholecalciferol acetate, only adducts to the exocyclic double bond were formed.[15]

Copper-catalyzed [2+3] dipolar cycloaddition of abietic acid propargyl ester derivatives with azides afforded quaternary ammonium compounds exhibiting strong antibacterial and antibiofilm activities against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*.[16]

This activity induced the present preparation of new derivatives of abietic acid as potentially active compounds.

EXPERIMENTAL

Materials and physical measurements

Reagent-grade chemicals were used without further purification unless otherwise noted. The aldehydes were purchased from Aldrich. The hydroximinoyl chlorides were prepared from the corresponding aromatic aldehyde oximes and *N*-chlorosuccinimide (NCS) in *N,N*--dimethylformamide (DMF).[17] The spectra were obtained as follows: IR spectra on a JASCO FTIR-420 spectrometer, ^{1}H-NMR spectra and ^{13}C-NMR spectra on Varian 500 Unity plus-500 and Varian 300 Unity plus 300 spectrometers in deuterated chloroform using TMS as the internal standard. 2D HSQC (heteronuclear single quantum coherence) and 2D HMBC (heteronuclear multiple bond correlation) analyses were realized on a Bruker Avance III 500 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane (TMS) as the internal standard; coupling constants are reported in Hz. In the ^{13}C-NMR spectra, signals of fluorine-substituted carbon atoms and some alpha carbon atoms were sometimes not observed because of strong [19]F–[13]C coupling. EI mass spectra were run on an AMD M-40 instrument. Flash chromatography was performed using silica gel S 230–400 mesh (Merck) and hexanes–ethyl acetate mixtures as eluents. Elemental analyses (C, H, N) were conducted on an elemental analyser XBO and the results were found to be in good agreement (±0.3 %) with the calculated values.

Methyl abietate (**2**) was prepared in 80 % yield. The ^{1}H- and ^{13}C-NMR spectra were found to be identical with the ones described in the literature.[18]

Analytical and spectral data of prepared compounds are given as Supplementary material to this paper.

General procedure for the preparation of the esters (3, 4 and 5)[19]

N,N-Dicyclohexylcarbodiimide (DCC) (1.72 g, 8.34 mmol) in dry CH_2Cl_2 was added under stirring at room temperature to a solution of abietic acid (6.3 mmol), alcohol (6.7 mmol) and 4-(dimethylamino)pyridine (0.196 g, 1.6 mmol) in a mixture of dry dichloromethane/acetonitrile (5 cm³, 1:1) under dry argon. Stirring was continued for 24 h. The reaction mixture was filtered and the filter paper washed with dichloromethane. The solution was washed with water, dilute HCl, water, an aqueous solution of sodium bicarbonate, and finally several times with water. The solution was dried ($MgSO_4$) and the product obtained after evaporation of the solvent was purified by flash chromatography on silica gel affording the expected ester **3**, **4** or **5** as yellowish waxes (35–80 %).

Phenyl abietate (**3**) was prepared in 45% yield. The ¹H- and ¹³C-NMR spectra were found to be identical with those described in the literature.[20]

Cycloaddition reaction of dipolarophiles 2, 3, 4 and 5 with 4-(trifluoromethyl)benzonitrile oxide (7a) and 4-isopropylbenzonitrile oxide (7b). A general procedure for the preparation of 8a, 8b, 9, 10, 11 and 12. 4-(Trifluoromethyl)benzonitrile oxide (**7a**) was generated as follows: a solution of the corresponding chloroxime (0.25 g, 1.12 mmol) in dry dichloromethane was passed through an Amberlyst-21 column. It was added dropwise over 30 min to the solution of a dipolarophile in dry dichloromethane, and the solution was stirred overnight at room temperature. Water was added, the organic layer separated and the aqueous one extracted with dichloromethane. The combined organic layers were dried ($MgSO_4$) and the product was purified by flash column chromatography.

Fungicidal testing

The compounds were screened *in vitro* for antifungal activity against the following five plant pathogens: *Fusarium culmorum* (W. G. Sm) Sacc., *Phytophthora cactorum* (Lebert & Cohn) Schroet, *Alternaria alternata* (Fr.) Keissl., *Rhizoctonia solani* J. G. Kühn and *Botrytis cinerea* Pers. ex Fr.

The test involved determination of mycelial growth retardation in potato glucose agar (PGA; Difco). Stock solutions of the test chemicals in acetone (2 cm³) were added to agar medium to give a concentration of 200 mg cm⁻³ and dispersed into sterile Petri dishes. Plates were inoculated within 24 h after they were poured. Four discs (5 mm diameter) were cut from the margins of an actively growing 2-week-old colony and placed equally distant from each other on the surface of the solidified agar. PGA with addition of acetone was used for the control. The plates were incubated in a growth chamber at 25±1 °C.

The radial growth of the fungal colonies was measured after 4–5 days depending on the growth rate of the control samples. The growth was determined by calculating the mean of two colony diameters of four replicate colonies. The fungicidal activity was expressed as the percentage of fungi growth inhibition compared to that of the untreated control. The relative growth inhibition of the treatment compared to the control was calculated as percentage, using the following formula:

$$\text{Inhibition, \%} = 100((x-y)/x)$$

where, x = fungal colony diameter in the control (mm) and y = fungal colony diameter in the treatment.

RESULTS AND DISCUSSION

Synthesis of the dipolarophiles

Methyl abietate (**2**) was obtained in a sulfuric acid-catalyzed esterification.[18] Phenyl abietate (**3**), benzyl abietate (**4**), and perillyl abietate (**5**) were prepared in the presence of dicyclohexylcarbodiimide and 4-dimethylaminopyridine (DCC/ /DMAP);[19] a side product was a corresponding urea derivative **6** formed in the reaction of abietic acid with DCC (Scheme 1).
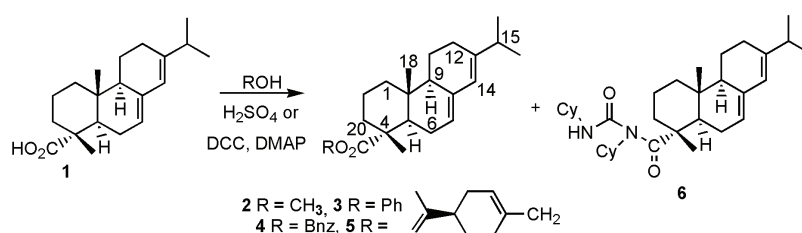

Scheme 1. Synthesis of the dipolarophiles.

The [2+3] cycloaddition reactions of benzonitrile oxides **7a** and **7b** to abietic acid esters **2–5** (Schemes 2–4) were investigated. The reaction of methyl abietate (**2**) with 4-(trifluoromethyl)benzonitrile oxide **7a** afforded a single *endo* adduct **8a** to the more exposed C7–C8 double bond. On the other hand, the reaction of nitrile oxide **7b** resulted in the *exo* adduct **8b** (Scheme 2).
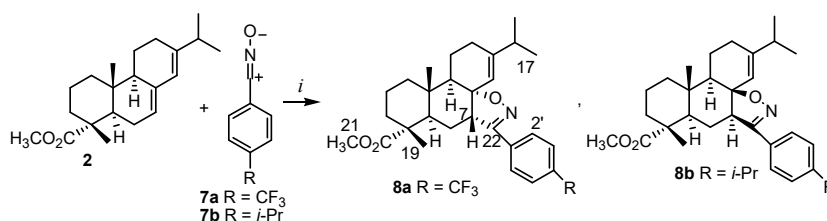
Scheme 2. Cycloaddition of dipoles **7a** and **7b** to methyl abietate (**2**). (*i*) CH₂Cl₂–CH₃CN, rt.

Cycloaddition reaction of nitrile oxide **7a** to the phenyl ester **3** also yielded a single *endo* adduct **9** to the same double bond, similarly as the addition of this dipole to the benzyl ester **4** (Scheme 3).

Finally, the [2+3] cycloaddition reaction of nitrile oxide **7a** to perillyl abietate (**5**) afforded a mixture of two bisadducts **11** and **12** to the exocyclic and C7––C8 double bonds (Scheme 4).

The structures of the dipolarophiles and cycloadducts were elucidated based on published data (for esters **2** and **3**[18,21]) and application of 1D and 2D ¹H-/¹³C--NMR spectroscopy.
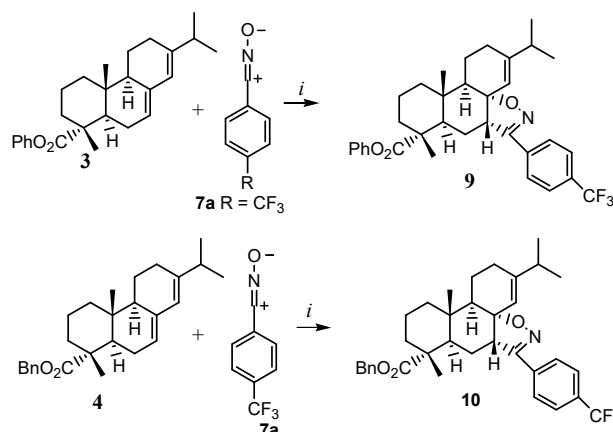
Scheme 3. Cycloaddition of dipole **7a** to phenyl abietate (**3**) and benzyl abietate (**4**).
(*i*) CH$_2$Cl$_2$–CH$_3$CN, rt.
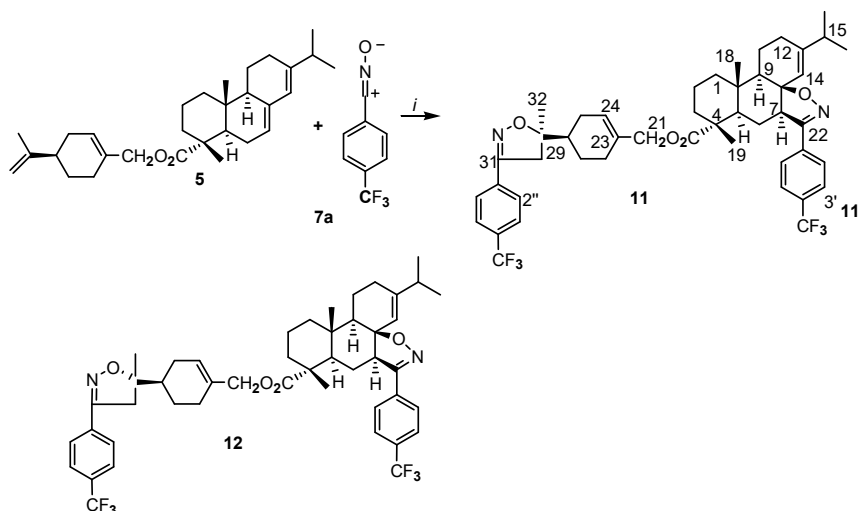
Scheme 4. Cycloaddition of dipole **7a** to perillyl abietate (**5**). (*i*) CH$_2$Cl$_2$–CH$_3$CN, rt.

The observed site selectivity of the reaction was proved by observing cross peaks in the 2D NMR HMBC spectra between the hydrogens of the isopropyl group and proximate C13–C14 carbon atoms. In the mono adducts, the following correlations were found between H15 at 2.17 ppm and H16/H17 at 0.88 ppm with C13 at 149.0 and C14 at 118.9 ppm in the adduct **8a**, H15 at 2.82 ppm and C13 at 145.8 ppm and C14 at 124.1 ppm in the adduct **8b**. Such interactions would not be possible in case of an alternative addition direction.

Similar cross peaks were found in bis-adducts between H16/H17 (1.22 ppm) and C13 (146.5 ppm) of compound **11,** as well as between H16/H17 (1.22 ppm) and C13 (145.8 ppm) in the diastereoisomer **12** (Fig. 1). A high regioselectivity

of the addition displayed in Schemes 2–4 was favored by orbital factors. The reaction of conjugated alkenes and alkenes with electron-donating substituents are controlled by $LUMO_{dipole}$–$HOMO_{dipolarophile}$ interactions and the oxygen atom of the dipole tends to attack the more substituted carbon atom of the dipolarophile.[21]
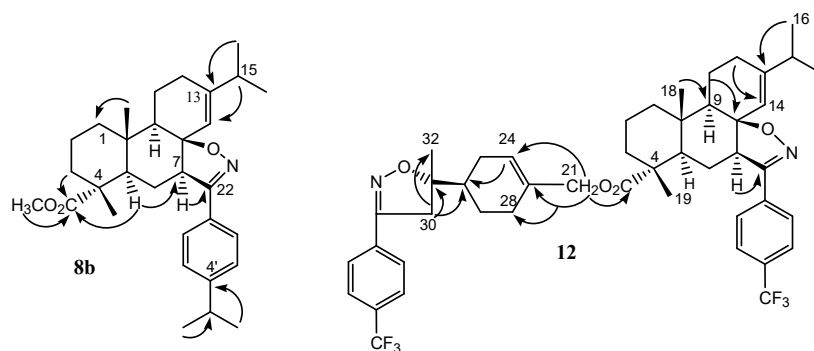

Fig. 1. Selected HMBC for adducts **8b** and **12**.

Reaction of nitrile oxides **7a** and **b** with abietic acid esters resulted in the formation of *endo* cycloadducts **8a**, **9** and **10** as well as *exo* cycloadducts **8b**, **11**, and **12**. These assignments were based first on the chemical shift of diagnostic angular C18 methyl groups that in *exo* isomers are deshielded by the C22 phenyl ring (δ 1.24–1.26 ppm and 16.3–17.3 ppm in the ^{13}C-NMR spectra). In *endo* isomers, the corresponding ^{1}H absorption is found at a higher field (0.81–0.86 ppm) and the ^{13}C absorption occurs at 14.1–15.3 ppm.

The resonances of bridgehead H7 protons are also diagnostic. In the *endo* products, absorption at a lower field was observed (at δ 3.14–3.68 ppm) than in the *exo* adducts (at δ 2.86–2.94 ppm). The lower chemical shifts of H7 in the *exo* adducts result from shielding by 1,3-diaxial interactions with H5 and H9 protons, and a weaker deshielding by the *anti* β-effect of the isoxazoline oxygen atom than the deshielding by the *syn* β-effect of this signal in the *endo* isomers (Figs. 1 and 2).[22] The signal of the H7 proton was unequivocally identified with the help of the 2D NMR HMBC spectra showing cross peaks of H7 and isoxazoline C22 carbon atom (2.92 and 154.2 for the **8b** adduct, 2.94 and 154.9 ppm for compound **11**, and 2.86 and 154.7 ppm for the bisadduct **12**.

The stereochemistry at C29 of the bisadducts was tentatively proposed and can be reversed.

The *in vitro* biological activity of the cycloadducts **8a**, **11**, **12** and dipolarophiles **2** and **4**–**6** against 5 fungal strains, common pathogens of important cultivated plants, was examined. The analyzed compounds showed only moderate fungicidal potency. From the abietic acid esters, benzyl abietate (**4**) was the most

active, particularly against *P. cactorum*. From the cycloadducts, bisadduct **11** displayed a higher potency than its C29 epimer **12** (Table I).

8b R = Me, R₁ = i-Pr ; **11**, **12** R = perillyl. R₁ = CF₃

8a R = Me, R₁ = CF₃; **9** R = Ph, R₁ = CF₃
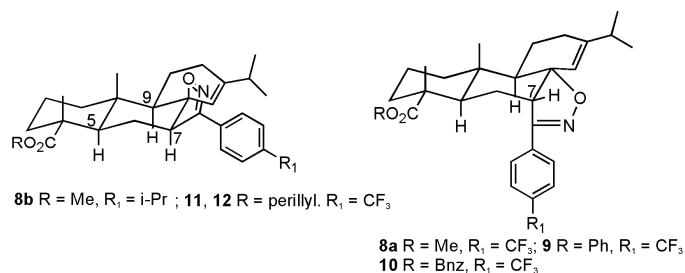**10** R = Bnz, R₁ = CF₃

Fig. 2. Structure of *exo* and *endo* isomers of compounds **8a**, **8b** and **10–12**.

TABLE I. Fungicidal inhibitory activity (linear growth inhibition, %) of compounds **2**, **4–6**, **8a**, **11** and **12** at 200 μg cm^{-3}

Compound	Fungus				
	Alternaria alternata	*Boritis cinerea*	*Fusarium culmorum*	*Phytophtora cactorum*	*Rhizoctonia solani*
2	–	9.0	23.0	3.0	2.0
4	30.0	36.0	0	47.0	30.0
5	36.0	10.0	0	0	36.0
6	0	0	0	0	26.0
8a	–	0	38.0	4.8	–
11	16.7	32.0	20.0	32.5	0
12	0	8.0	22.0	0	0
Chlorothalonil[a]	–	80.0	38.0	61.0	88.0

[a]Reference compound

CONCLUSIONS

High regio- and site selectivity of the [2+3] cycloaddition reaction of benzo-nitrile oxides to methyl, phenyl and benzyl abietates was found. Only mono adducts to the more exposed C7–C8 double bond were observed. On the other hand, the reaction with perrilic abietate afforded a mixture of bisadducts to the exocyclic and C7–C8 double bond. Some abietates and cycloadducts showed moderate biological activity.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of the synthesized compounds are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

И З В О Д

ИСПИТИВАЊЕ РЕАКЦИЈЕ [2+3] ЦИКЛОАДИЦИЈЕ НИТРИЛ-ОКСИДА И АБИЈЕТИНСКЕ КИСЕЛИНЕ

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Испитивана је реакција [2+3] циклоадиције ароматичних нитрил-оксида. Резулати су показали потпуну положајну селективност и региоселективност, док стереоселективност зависи од структуре алкена.

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