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The synthesis and the structure elucidation of *N,O*-diacetyl derivative of cyclic 3-hydroxymelatonin

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Abstract: Melatonin was subjected to an oxidation to give 3-hydroxymelatonin. All spectroscopic data for this compound were collected. *Ab initio* calculations for both possible configurations were performed. X-ray data on *N*,*O*-diacetyl derivative of 3-hydroxymelatonin allowed the unambigous structure determination.

Keywords: antioxidants, alkaloids, anti-cancer activity

1 Introduction

Among a large family of phytochemicals, compounds based on the 2,3,8,8a-tetrahydropyrrolo[2,3-b]indole heterocyclic system are extremely valuable because of their therapeutic potential in human and animal diseases [1]. In particular, two groups of compounds seem

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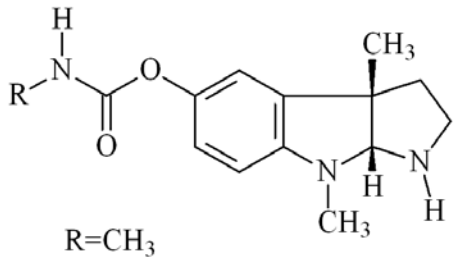
to be of special interest for pharmacochemistry.

Anticholinoesterases, such as physostigmine **1** (Scheme 1a), isolated from Calabar beans, along with its arylcarbamate analogues have proven to be promising drugs in the treatment of a variety of neurological disorders related to cholinergic transmission irregularities, e.g., Alzheimer's disease [2].



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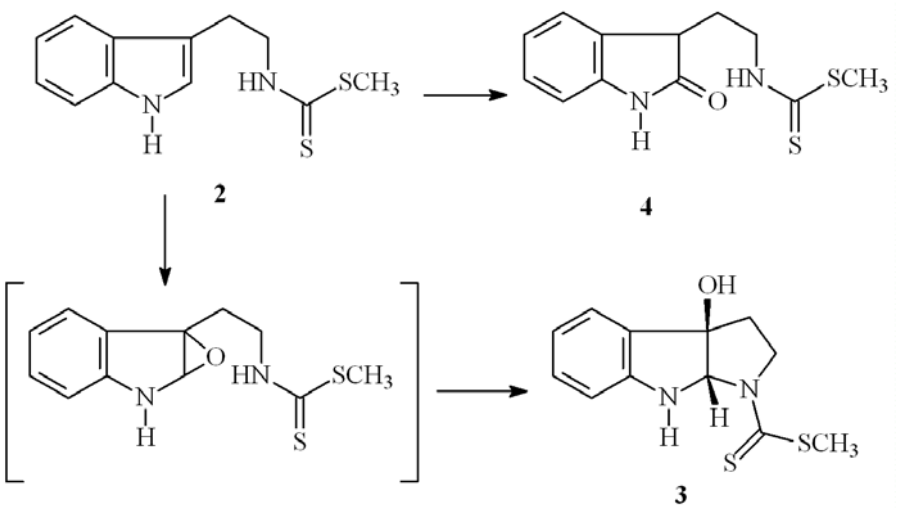
Scheme 1a

The second series of compounds known as phytoalexins, have recently been the subject of intensive pharmacochemical examination. Phytoalexins are secondary metabolites synthesized in plants in response to biotic or abiotic stress [3]. Some of them have been positively tested against leukemia and melanoma in mice [4]. Especially, the indole-derived phytoalexins have been found to possess significant antiproliferative activity against various cancer cells [5]. It has been documented that certain phytoalexins after in vivo cytochrome P450 oxidation are transformed into potent antileukemic agents [6].

The oxidation process has shown an important detoxification mechanism of phytoalexins by involving pathogenic fungi [7]. When a brassinin homologue **2** was subjected to a fungal oxidation process, several metabolites were formed, including 3a-hydroxycompound **3** and 2-oxindole derivative **4** [7] as presented in Scheme 1b.



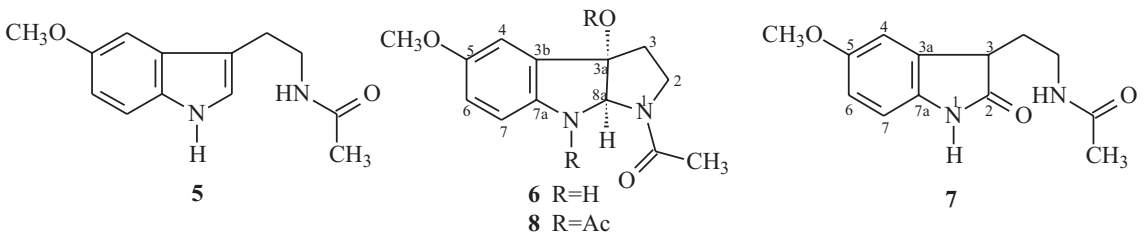
Scheme 1b

Free radicals are generated in vivo and then oxidatively damage DNA because of their

high reactivities. This action is attributed to several different kinds of diseases including cancer and Alzheimer's [8]. Melatonin 5 (N-acetyl-5-methoxytryptamine), presented in Scheme 1c, an endogenously produced indole found throughout the animal kingdom, was recently reported to be an effective scavenger of a number of reactive oxygen and nitrogen containing species both in vitro and in vivo [8].

2 Results and discussion

In numerous investigations melatonin **5** has been shown to be oxidized, e.g., by hydroxyl radicals derived from Fenton-type reagents or from light-induced homolysis of hydrogen peroxide [9]. Singlet oxygen oxidation of melatonin **5** results in different products, depending on the conditions applied [10]. Among a vast array of possible chemical structures of the products formed, two of them seem to be worth noting because of their strong resemblance to the already mentioned compounds **3** and **4**. We found that substantial amounts of (3aS*,8aR*)-1-acetyl-5-methoxy-2,3,8,8a-tetrahydropyrrolo[2,3-*b*]indol-3a(1*H*)-ol **6** and 5-methoxy-3-(N-acetyl)-ethylamino-2-oxindole **7** [11] were present in post-oxidation mixtures of melatonin **5** (Scheme 1c).



Scheme 1c

In particular, (3aS*, 8aR*)-1-acetyl-5-methoxy-2,3,8,8a-tetrahydropyrrolo[2,3-*b*]indol-3a(1*H*)-ol, also called cyclic 3-hydroxymelatonin **6** seems to be of interest due to its probable *in vivo* formation as a product of two hydroxyl radicals being scavenged by melatonin. Therefore the role of cyclic 3-hydroxymelatonin **6** as an anti-cancer agent has been seriously considered [12]. Furthermore, it is also linked to the inhibition of Alzheimer β-amyloid fibril formation by the oxidation products of melatonin **5** [13].

Because of the findings discussed above, we performed a more detailed structural investigation of cyclic 3-hydroxymelatonin **6**. Compound **6** was prepared according to the modified procedure of Nakagawa et al. [10]. Thus, melatonin **5** was subjected to the singlet oxygen treatment (O_2, halogen lamp, Bengal rose, MeOH, -78^{o}C). After decomposition of per-oxy compounds with dimethyl sulphide (Me_2S), cyclic 3-hydroxymelatonin **6** was isolated and purified by column chromatography and yielded an amorphous substance. Compound **6** and its several derivatives are currently under intensive biochemical investigations.

The results of ^{1}H NMR analysis are consistent with those reported by Nakagawa et al. [10]. In order to accumulate more precise analytical data than that available

from the seventies, we recorded the HETCOR spectra so as to conclusively identify all characteristic signals. The most indicative signal for the cyclic structure of C8a-H was identified at 5.30 ppm in ^{1}H NMR and was correlated with 82.40 ppm resonance in ^{13}C NMR accordingly (Figure 1).

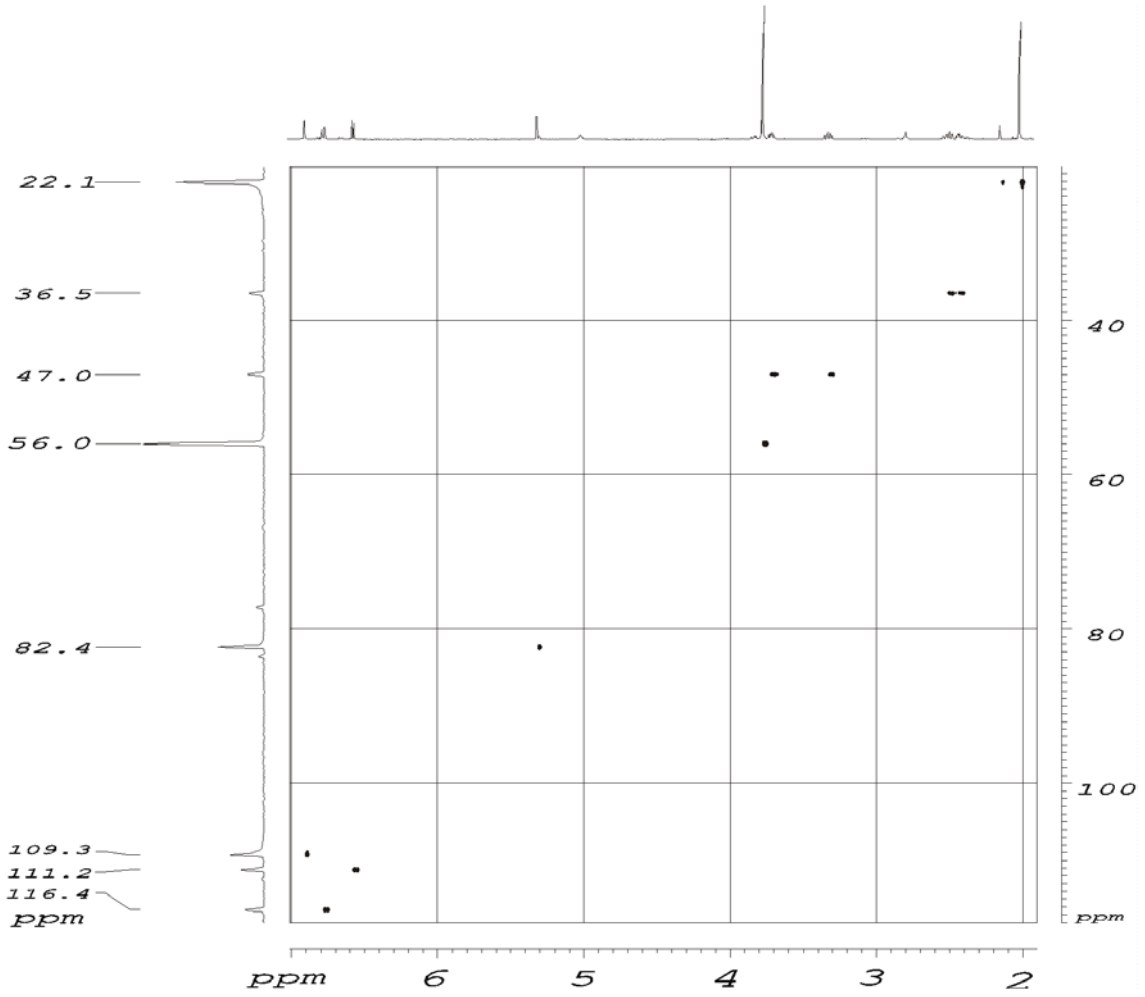


Fig. 1 The HETCOR spectrum of 3-hydroxymelatonin **6**.

All attempts to produce a stable solid derivative of **6** initially failed, so it was transformed into its N,O-diacetylderivative **8** (Scheme 1c) [10]. After an extensive effort, a monocrystal suitable for an X-ray analysis was obtained.

When the ^{1}H NMR spectrum of **8** was compared with that in the literature, virtually all identical peaks were found, including the signal attributed to C8a-H at 6.33 ppm that corresponded to the signal at 6.30 ppm as reported by Nakagawa [10]. Also, collecting DEPT 135 was helpful since it allowed us to distinguish signal C3a (90.19 ppm) from signal C9a (79.94 ppm).

Both spectral analysis and the results of an X-ray study unambiguously proved the structure of **8** with cis-juction of the rings B and C in the molecule. We would like to point out that the problem of the initial formation of the cis-type relative stereochemistry at C3a and C8a in compound **6** is not obvious.

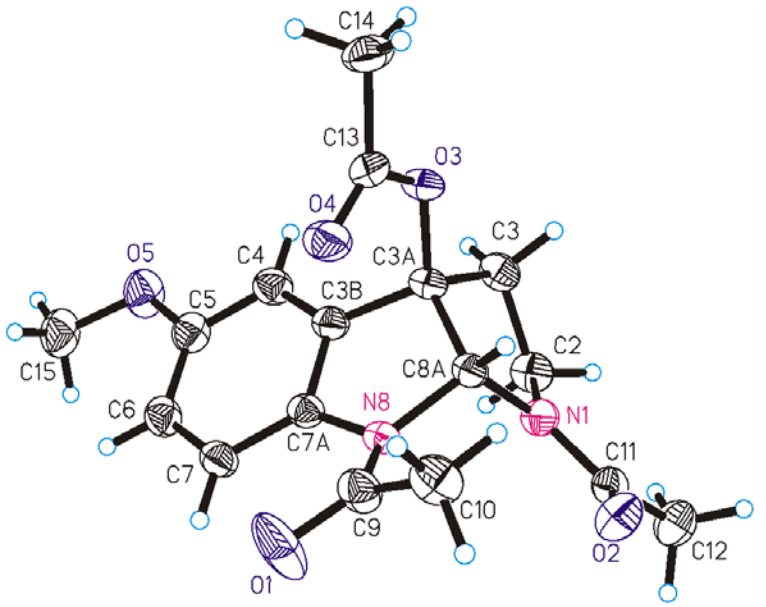


Fig. 2 ORTEP diagram for N,O-diacetylderivative **8**.

As was already demonstrated by Brossi and Greig [2] in a similar system of physostigmine derivatives, the resulting compound might exist both in the cis– and trans–diastereomeric forms, with the cis-form being responsible for 75 % of the population of stereoisomers. The preponderance of the cis form was also concluded from the calculations of the steric energy differences and was finally confirmed by the X-ray crystallography. The conformation of **8** is shown in Figure 2. All bond distances and angles have generally acceptable values. In the crystal there are no strong hydrogen bonds present. The detailed structural parameters have been deposited with Cambridge Structural Data Centre under the number CCDC 224690.

We also performed ab initio Hartree-Fock calculations of the energy differences for the cis and trans isomers of **6**. PC Spartan Pro software was used in all quantum chemical calculations [14]. Stationary points have been verified by Hessian matrix calculations. Based on energy calculations, we obtained an ΔE=-20,3 kcal/mol, thus again favoring the cis isomer of **6** (Figure 3).

Fig. 3 The RHF/6-31G** calculations of cis and trans (3aSR, 8aRS)-1-acetyl-5-methoxy-2,3,8,8a-tetrahydropyrrolo[2,3-*b*]indol-3a(1*H*)-ol.

Melatonin **5** was also subjected to another oxidation experiment with m-chloroperbenzoic acid (mCPBA). We found that melatonin was consumed completely with simultaneous formation of 3-hydroxymelatonin **6** (5 %), 5-methoxy-3-(N-acetyl)-ethylamino-2-oxindole **7** (30 %), AFMK [11] (30 %) and several compounds which have not been identified. The comparison of the spectral data that we obtained for compounds **6** and **7** with those available in literature [9, 11, 15] suggests that the spectral assignments for **6** given in [14] resemble very closely the data provided by Dryhurst [11] for compound **7**. This problem will be the subject of a separate study in the future.

It may be concluded that the structure of 3-hydroxymelatonin **6** seems to be of considerable interest taking into account the fact that several comparable skeleton types are present in plant and animal kingdoms and play very important roles in modern pharmacochemistry.

3 Experimental

The NMR spectra were recorded on a Varian Unity Plus operating at 500 MHz and a Bruker DRX 500 operating at 500 MHz spectrometers. The GC-MS analyses were carried out on a HP 6890 Plus gas chromatograph coupled to HP 5973 mass detector. Melting point of compound **8** was measured on Kofler apparatus (type Boetius). X-ray crystallographic data were collected at T=293 K on a Kuma KM4 diffractometer with Mo-K_α radiation. Structure was solved using direct methods from SHELXS-97 program and refined by application of SHELXL-97 software [16]. Melatonin was obtained as a gift from LEK-AM Sp. z o.o. Pharmaceutical Company, Poland. The gift is kindly acknowledged by ZC.

3.1 Preparation of cyclic 3-hydroxymelatonin 6

Cyclic 3-hydroxymelatonin was prepared according to the modified procedure provided by Nakagawa by omitting the use of the 480 nm $< h\nu$ filter [10]. Thus, a sample of 2.15 g of melatonin was dissolved in 200 mL of dry methanol containing 2 mL of dry pyridine and 60 mg of Rose Bengal dye (Sigma-Aldrich Sp. z o.o., Poznań, Poland) and the flask was immersed in acetone/dry ice bath. Air was replaced by oxygen and the mixture was irradiated with 400 W halogen lamp for 8 h with vigorous stirring. Thereafter, the irradiation was terminated and oxygen was replaced by argon; 10 mL of dimethyl sulfide were added and the mixture was allowed to reach room temperature overnight. After evaporation under reduced pressure the residue was dissolved in a minimum amount of dichloromethane and was subjected to a column chromatography on alumina (Merck, basic alumina, activity III according to Brockmann). Elution with chloroform allowed the recovery of unreacted melatonin (2.05 g). Subsequent elution with 3 % (v/v) methanol in dichloromethane gave the title cyclic 3-hydroxymelatonin **6** as amorphous solid in 95 mg yield. ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 1.99 s, 3H ($COCH_3$) 2.40 m, 1H (C3-H), 2.47 m, 1H (C3-H), 3.30 m, 1H (C2-H), 3.6 v. brs, 2H (NH and OH), 3.70 m, 1H

(C2-H), 3.75 s, 3H (OCH_3), 5.30 s, 1H (C8a-H), 6.56 d, J=8.5Hz, 1H (C7-H), 6.75 dd, J=8.5+ 2.0Hz, 1H (C6-H), 6.89 d, J=2.0Hz, 1H (C4-H). ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm): 22.06 ($CO\underline{C}H_3$), 36.53 (C3), 47.08 (C2), 56.02 (OCH_3), 82.40 (C8a), 86.96 (C3a), 109.33 (C4), 111.50 (C7), 116.32 (C6), 130.65 (C3b), 142.95 (C7a), 153.94 (C5), 170.79 (CO). EI-MS: m/z (rel. intensity in %) 248 (M^+, 100), 233 (13), 220 (3), 205 (6), 191 (8), 178 (28), 162 (14), 150 (9), 133 (8). HR MS ES(+): calc. for $C_{13}H_{17}N_2O_3$249,1239 found 249,1241. HETCOR ^{1}H-^{13}C (500 MHz, $CDCl_3$). Spectrum width = 4166.67 Hz, 2D width = 25,000 Hz, acquisition time = 0.246 s, relaxation delay = 1.2 s, repetitions = 2, increments = 2 x 1024, pulseprogram: invietgs.

3.2 Preparation of N,O-diacetyl derivative of cyclic 3-hydroxymelatonin **8**

A sample of 30 mg cyclic 3-hydroxymelatonin **6** was dissolved in 10 mL of dry pyridine to which 0.5 mL of acetic anhydride was introduced at 0 ^{o}C under argon atmosphere and the mixture was stirred at the same temperature overnight. After removal of the volatile components at reduced pressure, the residue was taken up into toluene (10 mL). The organic layer was washed consecutively with water, 1 % citric acid and brine. After drying over anhydrous magnesium sulfate and evaporation of the solvent, the residue was purified by column chromatography on silica gel. Elution with 3 % (v/v) methanol in ethyl acetate afforded compound **8** in 77 % yield in the form of white crystals. Mp: 159-160 ^{o}C (EtOH). Lit. mp 156-158 ^{o}C (EtOH) [10].^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 2.58 + 2.08 + 2.03 three s, 9H ($COCH_3$), 2.49 m, 1H (C3-H), 2.84 m, 1H (C3-H), 3.09 m, 1H (C2-H), 3.73 m, 1H (C2-H), 3.81 s, 3H (OCH_3), 6.33 s, 1H (C8a-H), 6.92 dd, J=9 + 2.5Hz, 1H (C6-H), 7.07 d, J=2.5Hz, 1H (C4-H), 8.00 d, J=9Hz, 1H (C7-H).^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm): 23.43 + 22.72 + 21.37 (three $CO\underline{C}H_3$), 36.02 (C3), 46.59 (C2), 55.75 (OCH_3), 79.94 (C8a), 90.19 (C3a), 110.25 (C6), 116.07 (C4), 119.86 (C7), 129.59 (C3b), 138.29 (C7a), 156.83 (C5), 171.20 + 170.09 + 169.65 (three CO). HR MS ES(+): calc. for $C_{17}H_{20}N_2O_5Na$ 355,1270 found 355,1271.

It was found that N,O-diacetyl-3-hydroxymelatonin **8** crystallizes in the centrosymmetric $P2_1/n$ space group with Z=4 molecules in the unit cell [$F(000)$=704]. The unit cell parameters have been determined by the least squares treatment of 32 strong reflections found within $12<2\theta<24^o$ using $MoK\alpha$ radiation [$\lambda(MoK\alpha)$=0.71073 Å]: a=8.3770(17), b=16.311(3), c=12.006(2) Å, β=100.49(3)^o. 5592 data were collected up to $2\theta=60^o$. The final R and wR values were of 0.0518 and 0.1582, respectively for 1894 observed reflections with $I>2\sigma(I)$.

3.3 Preparation of 5-methoxy-3-(N-acetyl)-ethylamino-2-oxindole **7**

A sample of 150 mg (0.6 mM) melatonin **5** was dissolved in 50 mL of dry dichloromethane to which 280 mg (1.6 mM) of m-chloroperbenzoic acid was added while stirring. After 0.5 h the reaction was terminated by washing with sodium hydrosulfite ($NaHSO_3$, 10 %). The organic layer was then washed with water and dried with magnesium sulfate. Subsequent

filtering through alumina (Merck, basic alumina, activity IV according to Brockmann) with dichloromethane and evaporating under reduced pressure gave 130 mg of an oil. The crude mixture was then purified by column chromatography on silica gel (Merck, 100-200 mesh). Elution with 1 % (v/v) methanol in dichloromethane yielded 53 mg (30 % chemical yield) of a yellow oil of **7**.^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 1.96 s, 3H ($COCH_3$), 2.23 + 2.04 two m, 2H (CH_2-C$\underline{H}_2$-NH-), 3.48 m, 2H (C$\underline{H}_2$-CH_2-NH-), 3.48 m, 1H (C3-H), 3.79 s, 3H (OCH_3), 6.38 brs, 1H (NH-side chain, exchangeable with D_2O), 6.75 dd, J=8.5 + 2.0Hz, 1H (C6-H), 6.79 d, J=8.5Hz, 1H (C7-H), 6.90 d, J=2.0Hz, 1H (C4-H), 8.21 s, 1H (N1-H, exchangeable with D_2O). ^{1}H NMR (500 MHz, CD_3CN), δ (ppm): 1.81 s, 3H ($COCH_3$) 2.15 m, 2H (CH_2-C$\underline{H}_2$-NH-), 3.30 + 3.22 two m, 2H (C$\underline{H}_2$-CH_2-NH-), 3.40 t, J=6.0Hz, 1H (C3-H), 3.75 s, 3H (OCH_3), 6.59 brs, 1H (NH-side chain), 6.75 dd, J=9.0 + 1.0Hz, 1H (C6-H), 6.80 d, J=9.0Hz, 1H (C7-H), 6.96 d, J=1.0Hz, 1H (C4-H), 8.39 s, 1H (N1-H). ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm): 23.25 (CO$\underline{C}H_3$), 30.03 (C3), 37.20 ($\underline{C}H_2$-CH_2-NH-), 44.82 (CH_2-$\underline{C}H_2$-NH-), 55.84 (OCH_3), 110.16 (C4), 111.18 (C7), 112.95 (C6), 130.52 (C7a), 134.31 (C3a), 156.07 (C5), 170.46 ($\underline{C}OCH_3$), 180.04 (C2). EI-MS: m/z (rel. intensity in %) 248 (M^+, 100), 230 (3), 206 (13), 190 (50), 176 (100), 160 (13), 133 (8). HR MS ES(+): calc. for $C_{13}H_{17}N_2O_3$ 249,1239 found 249,1242.

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