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SYNTHESIS OF (*Z*)- AND (*E*)-3-(2-CHLOROPYRIDIN-5-YLMETHYL) OXIMINO-(22*E*,24*R*)-ERGOSTA-4,7,22-TRIENES AND THEIR OXIDATIVE TRANSFORMATIONS

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*(Z)- and (*E*)-3-(2-chloropyridin-5-ylmethyl)oximino-(22*E*,24*R*)-ergosta-4,7,22-trienes (**5**-**6**) were synthesized by chemical transformation of ergosterol. Several oxidative transformations of them were studied. It was found that oxidation of these compounds by chromium(VI) oxide formed the corresponding* O*-substituted 3-ketoximes of the mycosteroid (22*E*,24*R*)-ergosta-4,7,22-trien-3,6-dione (7) and (**8**), which contained* α*-chloropyridine fragments characteristic of biologically active neonicotinoids. It was shown that oxidation of **5** and **6** by selenium dioxide occurred with formation of the corresponding 9*α*-hydroxy derivatives **9** and **10**.*

Keywords: (*Z*)- and (*E*)-3-(2-chloropyridin-5-ylmethyl)oximino-(22*E*,24*R*)-ergosta-4,7,22-trienes, *O*-substituted 3-ketoximes of (22*E*,24*R*)-ergosta-4,7,22-trien-3,6-dione, *O*-(2-chloropyridin-5-ylmethyl)hydroxylamine, synthesis.

We synthesized previously [1] a series of *O*-substituted 6-ketoximes of 3*β*,5-dihydroxy-6-ketosteroids. One of the characteristic features of the synthesized compounds was the presence in them of an *α*-chloropyridine group, which is typical of modern neonicotinoid insecticides [2] and the pharmacologically valuable alkaloid epibatidine [3]. Biological testing of these steroidal oximes discovered compounds with high insecticidal activity. This prompted us to continue research in this area, in particular, to synthesize analogous *O*-substituted 3-ketoximes of (22*E*,24*R*)-ergosta-4,7,22-trien-3,6-dione (**1**). Steroid **1** is a natural compound that was observed in the marine sponge *Raphidostila incisa* [4] and fungi *Ganoderma lucidum* [5, 6] and *Calvatia cyathiformis* [5, 7]. This compound can also be obtained by chemical synthesis from ergosterol **2** [8].

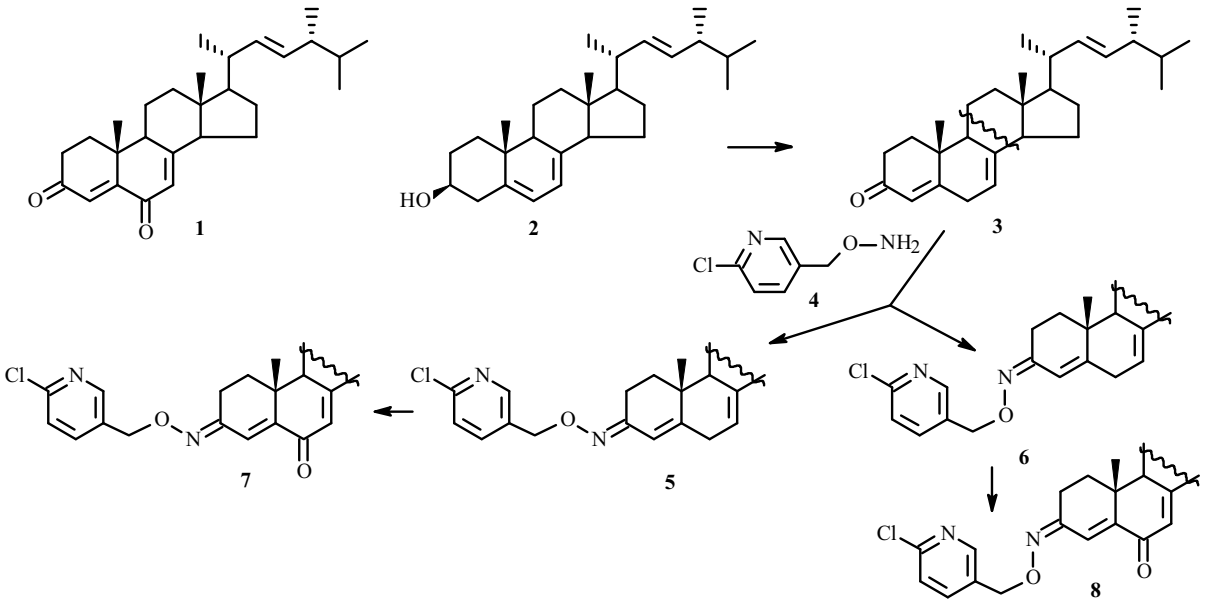

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TABLE 1. Chemical Shifts of C Atoms (CD_2Cl_2, δ, ppm) in ^{13}C NMR Spectra of **5**–**10**

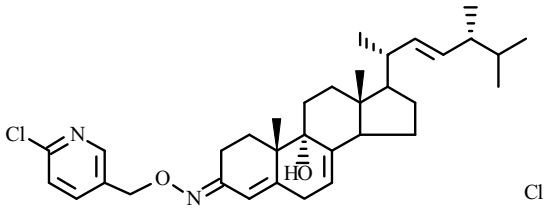
C atom	**5**	**6**	**7**	**8**	**9**	**10**
1	32.6	34.2	34.2	35.7	28.2	29.3
2	19.9	25.3	19.7	24.7	21.3	26.1
3	154.5	154.9	156.3	153.2	150.0	153.9
4	116.0	110.3	124.1	116.2	119.8	114.2
5	157.6	158.3	166.9	167.5	157.5	154.5
6	32.8	33.3	186.1	187.6	32.8	33.0
7	116.8	116.4	124.4	124.4	119.9	119.2
8	139.8	139.9	147.8	150.5	142.2	142.0
9	46.5	46.7	46.8	47.4	76.7	76.1
10	37.7	38.7	39.0	39.9	43.8	44.6
11	22.5	22.4	22.3	22.1	27.0	27.0
12	39.6	39.5	39.0	38.9	36.0	35.9
13	43.8	43.8	44.6	44.7	43.4	43.7
14	55.5	55.4	56.4	56.5	51.4	51.4
15	23.3	23.3	23.1	23.0	23.4	23.4
16	28.5	28.5	28.2	28.1	28.5	28.5
17	56.3	56.3	56.5	56.5	56.1	56.0
18	12.3	12.3	12.9	12.9	11.6	11.6
19	21.5	21.8	20.4	20.2	23.8	23.3
20	40.9	40.8	40.7	40.6	40.8	40.8
21	21.3	21.3	21.3	21.3	21.3	21.3
22	136.0	136.0	135.5	135.5	136.0	136.0
23	132.4	132.4	132.9	132.9	132.4	132.4
24	43.3	43.3	43.3	43.3	43.3	43.3
25	33.5	33.5	33.5	33.5	33.5	33.5
26	20.1	20.1	20.1	20.1	20.1	20.1
27	19.8	19.8	19.8	19.8	19.8	19.8
28	17.8	17.8	17.8	17.8	17.8	17.8
$-CH_2ON=$	72.5	72.2	73.4	72.9	72.5	72.3
$C\text{-}2_{Py}$	150.9	150.9		151.2	150.9	150.9
$C\text{-}3_{Py}$	124.3	124.3	125.4	124.2	124.3	124.3
$C\text{-}4_{Py}$	139.0	139.0	139.3	139.2	139.0	139.0
$C\text{-}5_{Py}$	133.7	133.7		133.2	133.6	133.6
$C\text{-}6_{Py}$	149.7	149.6	149.9	149.8	149.7	149.7

We used ergosterol **2** as the starting material for preparing the target compounds. Oppenauer oxidation [8] of **2** produced the 4,7,22-trien-3-one (**3**). Reaction of **3** with *O*-(2-chloropyridin-5-ylmethyl)hydroxylamine (**4**) in benzene formed two isomeric 3-ketoximes that were separated by column chromatography and isolated from the reaction mixture in yields of 49 and 39%, respectively.

The structure of the main reaction product was established using spectral data as (3*E*)-3-ketoxime (**5**); of the minor product, its (3*Z*)-isomer (**6**). The chemical shifts of the H-2 and H-4 resonances in PMR spectra of these compounds were very valuable for proving their structures. According to the literature [9], the spatial proximity of the electronegative O atom of the oxime causes a characteristic weak-field shift of the resonances in the PMR spectra for the H atoms that are sterically close to it compared with resonances of the analogous atoms in the other isomer. Thus, the oxime in **6** has the (*Z*)-geometry according to the chemical shift for H-4 (δ 6.41 ppm) (for comparison, the analogous resonance in the spectrum of oxime **5** has chemical shift δ 5.76 ppm). By analogy, the structure of **5** as the (*E*)-3-ketoxime could be determined by comparing chemical shifts of H-2α (δ 2.46 ppm) and H-2β (δ 2.57 ppm) resonances in PMR spectrum with those values in the spectrum of **6** (δ 2.19–2.29 ppm for H-2α and -2β).

In the next step, allylic oxidation at C-6 by chromium(VI) oxide in Py synthesized from **5** and **6** the corresponding 4,7,22-trien-3,6-diketone 3-ketoximes **7** and **8**, respectively. The structures of these followed unambiguously from the spectral data presented in Table 1 and the Experimental section.

Because of the low yield in the allylic oxidation of **5**, we attempted also to introduce the 6-ketone into **5** and **6** via oxidation by selenium dioxide. It was hypothesized that this reaction could synthesize the corresponding 6-hydroxy derivatives that could then be oxidized to the corresponding 6-ketones. However, it turned out that in both instances allylic oxidation by selenium dioxide occurred not at the more available C-6 or C-14 but at C-9 to form 9α-hydroxy derivatives **9** and **10**, respectively. The structures of these were determined by analyzing spectral data. In particular, the ^{13}C NMR spectra of these compounds (Table 1) showed a characteristic shift to weak field for the C-9 resonances that was caused by the α-effect of the hydroxyl as compared with the positions of the analogous resonances in starting **5** and **6**.



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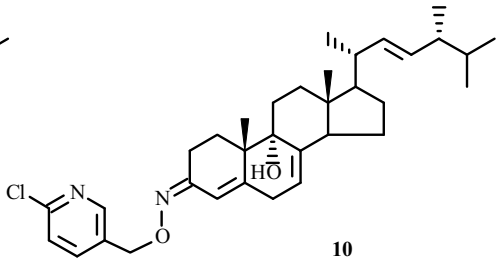

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EXPERIMENTAL

IR spectra were recorded on a Bomem–Michelson FTIR spectrometer in the range 700–3600 cm^{-1}. PMR and ^{13}C NMR spectra were taken on a Bruker Avance 500 NMR spectrometer (operating frequency 500.13 MHz for ^{1}H; 125.75, ^{13}C). Chemical shifts are given vs. TMS as an internal standard. Mass spectra were recorded on an Accela HPLC system with an LCQ-Fleet mass-detector (three-dimensional ion trap) using chemical ionization at atmospheric pressure (APCI) (detection of positive ions). The reactive gas was N_2. The *m*/*z* values are given for the strongest peaks. The course of reactions and purity of products were monitored using Kieselgel $60F_{254}$ plates (Merck). Melting points were determined on a Kofler block.

Oximation of (22*E*,24*R*)-Ergosta-4,7,22-trien-3-one (3). A solution of **3** (1.78 g, 4.51 mmol) (obtained via oxidation of **2** using the method of Oppenauer [8]) and **4** (0.87 g, 5.49 mmol) (prepared by the literature method [1]) in benzene (40 mL) was held at 12–15°C for 17 h, treated with $MgSO_4$ (2 g), and stirred for 1.5 h. The desiccant was filtered off. The filtrate was evaporated in vacuo. The solid was chromatographed over a column of silica gel with elution by petroleum ether (70–90°C):EtOAc mixtures of increasing polarity (from 50:1 to 20:1). The unseparated portion of the product was chromatographed again over a column of silica gel with elution by cyclohexane:EtOAc mixtures of increasing polarity (from 150:1 to 20:1) to afford (3*E*,22*E*,24*R*)-3-(2′-chloropyridin-5′-ylmethyloximino)-ergosta-4,7,22-triene (**5**) (1.18 g, 2.2 mmol), yield 49%, mp 107–110°C. IR spectrum (KBr, ν, cm^{-1}): 1636, 1587, 1568, 1460 (–C=C–C=N–).

PMR spectrum (CD_2Cl_2, δ, ppm, J/Hz): 0.59 (3H, s, 18-Me), 0.83 (3H, d, J = 8, 26/27-Me), 0.84 (3H, d, J = 8, 26/27-Me), 0.92 (3H, d, J = 7, 28-Me), 1.02 (3H, d, J = 7, 21-Me), 1.07 (3H, s, 19-Me), 2.46 (1H, ddd, J_1 = 17, J_2 = 9.5, J_3 = 5.0, H-2α), 2.57 (2H, m, H-6α, 2β), 3.04 (1H, br.d, J = 19, H-6β), 5.04 (2H, s, $–CH_2–O–N=$), 5.16-5.26 (3H, m, H-22, 23, 7), 5.76 (1H, d, J = 2, H-4), 7.30 (1H, d, J = 8, $H\text{-}3_{Py}$), 7.66 (1H, dd, J_1 = 8, J_2 = 2.5, $H\text{-}4_{Py}$), 8.34 (1H, d, J = 2.5, $H\text{-}6_{Py}$).

Mass spectrum (*m*/*z*): 536 $[M + 1]^+$.

Further elution isolated (3*Z*,22*E*,24*R*)-3-(2′-chloropyridin-5′-ylmethyloximino)-ergosta-4,7,22-triene (**6**) (0.93 g), yield 39%, mp 141–143°C. IR spectrum (KBr, ν, cm^{-1}): 1634, 1593, 1570, 1460 (–C=C–C=N–).

PMR spectrum (CD_2Cl_2, δ, ppm, J/Hz): 0.59 (3H, s, 18-Me), 0.83 (3H, d, J = 8, 26/27-Me), 0.84 (3H, d, J = 8, 26/27-Me), 0.92 (3H, d, J = 6.5, 28-Me), 1.02 (3H, d, J = 6.5, 21-Me), 1.09 (3H, s, 19-Me), 2.19–2.29 (2H, m, H-2α, 2β), 2.60 (1H, br.d, J = 19, H-6α), 3.07 (1H, br.d, J = 19, H-6β), 5.01 (2H, s, —$CH_2–O–N=$), 5.16–5.26 (3H, m, H-7, 22, 23), 6.41 (1H, d, J = 2, H-4), 7.30 (1H, d, J = 8.5, $H\text{-}3_{Py}$), 7.66 (1H, dd, J_1 = 8.5, J_2 = 2.5, $H\text{-}4_{Py}$), 8.34 (1H, d, J = 2.5, $H\text{-}6_{Py}$).

Mass spectrum (*m*/*z*): 536 $[M + 1]^+$.

(3*Z*,22*E*,24*R*)-3-(2′-Chloropyridin-5′-ylmethyloximino)-ergosta-4,7,22-trien-6-one (8). A mixture of CH_2Cl_2 (30 mL) and Py (3.75 mL, 46.25 mmol) was dried over 4-Å molecular sieves (150 mg) for 24 h. The resulting mixture was stirred, treated with chromium(VI) oxide (1.85 g, 18.5 mmol), stirred for 1 h, treated with **6** (0.2 g, 0.37 mmol), and stirred for another 25 min. The excess of oxidant was neutralized by 2-propanol (2.8 mL, 37 mmol). The precipitate was filtered off and washed on the filter with CH_2Cl_2:H_2O (1:1, 60 mL). The organic phase was separated from the combined filtrate. The

aqueous layer was extracted with CH_2Cl_2 (2 × 15 mL). The organic extracts were combined and washed with H_2O (3 × 25 mL) and dried over $MgSO_4$. The desiccant was filtered off. The filtrate was evaporated in vacuo. The solid was co-evaporated three times with toluene and chromatographed over a column of silica gel with elution by cyclohexane:EtOAc mixtures of increasing polarity (from 17:1 to 6:1) to afford (3*Z*,22*E*,24*R*)-3-(2′-chloropyridin-5′-ylmethyloximino)-ergosta-4,7,22-trien-6-one (**8**) (0.07 g, 0.127 mmol), yield 34%, mp 155–158°C [petroleum ether (70–90°C):EtOAc]. IR spectrum (film, ν, cm^{-1}): 1656, 1651, 1618, 1591, 1568, 1460 (–C=C–C=O, –C=C–C=N–).

PMR spectrum (CD_2Cl_2, δ, ppm, J/Hz): 0.67 (3H, s, 18-Me), 0.83 (3H, d, J = 8.5, 26/27-Me), 0.84 (3H, d, J = 8.5, 26/27-Me), 0.92 (3H, d, J = 7, 28-Me), 1.04 (3H, d, J = 6.5, 21-Me), 1.17 (3H, s, 19-Me), 2.34 (1H, ddd, J_1 = 11, J_2 = 7.5, J_3 = 2, H-9α), 2.38–2.41 (2H, m, H-2α, 2β), 5.09 (2H, s, $–CH_2–O–N=$), 5.19 (1H, dd, J_1 = 15.5, J_2 = 8, H-22/H-23), 5.26 (1H, dd, J_1 = 15.5, J_2 = 8, H-22/H-23), 5.87 (1H, t, J = 2, H-7), 7.26 (1H, s, H-4), 7.31 (1H, d, J = 8, $H\text{-}3_{Py}$), 7.68 (1H, dd, J_1 = 8, J_2 = 2.5, $H\text{-}4_{Py}$), 8.35 (1H, d, J = 2.5, $H\text{-}6_{Py}$).

Mass spectrum (*m*/*z*): 549 $[M]^+$.

(3*E*,22*E*,24*R*)-3-(2′-Chloropyridin-5′-ylmethyloximino)-ergosta-4,7,22-trien-6-one (7). A mixture of CH_2Cl_2 (30 mL) and Py (3.95 mL, 48.75 mmol) was dried over 4-Å molecular sieves (290 mg) for 24 h. The resulting mixture was stirred, treated with chromium(VI) oxide (1.95 g, 19.5 mmol), stirred for 1 h, treated with **5** (0.228 g, 0.43 mmol), and stirred for another 20 min. The excess of oxidant was neutralized by 2-propanol (3.0 mL, 39 mmol). The mixture was treated with H_2O (15 mL). The precipitate was triturated in the biphasic system, filtered off, and washed on the filter with CH_2Cl_2:H_2O (1:1, 60 mL). The organic phase was separated from the combined filtrate. The aqueous phase was extracted with CH_2Cl_2 (2 × 15 mL). The organic extracts were combined and washed with H_2O (3 × 25 mL) and dried over $MgSO_4$. The desiccant was filtered off. The filtrate was evaporated in vacuo. The solid was co-evaporated three times with toluene and chromatographed over a column of silica gel with elution by cyclohexane:EtOAc mixtures of increasing polarity (from 17:1 to 8:1) to afford (3*E*,22*E*,24*R*)-3-(2′-chloropyridin-5′-ylmethyloximino)-ergosta-4,7,22-trien-6-one (**7**) (0.018 g, 0.033 mmol), yield 7.7%, mp 169–173°C (hexane:EtOAc). IR spectrum (film, ν, cm^{-1}): 1657, 1650, 1612, 1590, 1568, 1460 (–C=C–C=O, –C=C–C=N–).

PMR spectrum (CD_2Cl_2, δ, ppm, J/Hz): 0.67 (3H, s, 18-Me), 0.83 (3H, d, J = 8, 26/27-Me), 0.84 (3H, d, J = 8, 26/27-Me), 0.92 (3H, d, J = 7, 28-Me), 1.05 (3H, d, J = 6.5, 21-Me), 1.14 (3H, s, 19-Me), 2.31 (1H, ddd, J_1 = 18, J_2 = 5, J_3 = 2.5, H-9α), 5.16 (2H, s, $–CH_2–O–N$), 5.19 (1H, dd, J_1 = 15.5, J_2 = 7.5, H-22/H-23), 5.26 (1H, dd, J_1 = 15.5, J_2 = 7.5, H-22/H-23), 5.87 (1H, t, J = 2, H-7), 6.79 (1H, s, H-4), 7.32 (1H, d, J = 8.5, $H\text{-}3_{Py}$), 7.69 (1H, dd, J_1 = 8.5, J_2 = 2.5, $H\text{-}4_{Py}$), 8.37 (1H, d, J = 2.5, $H\text{-}6_{Py}$).

Mass spectrum (*m*/*z*): 550 $[M + 1]^+$.

(3*E*,22*E*,24*R*)-3-(2′-Chloropyridin-5′-ylmethyloximino)-ergosta-4,7,22-trien-9α-ol (9). A solution of selenium dioxide (0.083 g, 0.748 mmol) in dioxane (5 mL) was stirred, treated in one portion with **5** (0.1 g, 0.187 mmol), stirred for 15 min, and filtered through a layer of Al_2O_3. The sorbent was washed successively with dioxane (5 mL) and CH_2Cl_2 (20 mL). The filtrate was evaporated to dryness in vacuo. The crystalline solid was dissolved in CH_2Cl_2 (30 mL) and washed successively with aqueous $NaHCO_3$ solution (5%, 2 × 15 mL) and H_2O (2 × 15 mL). The organic phase was dried over $MgSO_4$. The desiccant was filtered off. The filtrate was evaporated in vacuo. The product was isolated by successive crystallizations first from EtOAc and then from a petroleum ether:EtOAc mixture to afford **9** (0.036 g, 0.0653 mmol), yield 35%, mp 165–174°C.

PMR spectrum (CD_2Cl_2, δ, ppm, J/Hz): 0.59 (3H, s, 18-Me), 0.83 (3H, d, J = 8, 26/27-Me), 0.84 (3H, d, J = 8, 26/27-Me), 0.92 (3H, d, J = 7, 28-Me), 1.03 (3H, d, J = 6.5, 21-Me), 1.17 (3H, s, 19-Me), 2.67 (1H, ddd, J_1 = 19.5, J_2 = 4.5, J_3 = 3.5, H-6α), 3.06 (1H, br.d, J = 19.5, H-6β), 5.04 (2H, s, $–CH_2–O–N=$), 5.16–5.26 (3H, m, H-7, 22, 23), 5.95 (1H, d, J = 1.5, H-4), 7.30 (1H, d, J = 8.5, $H\text{-}3_{Py}$), 7.66 (1H, dd, J_1 = 8.5, J_2 = 2, $H\text{-}4_{Py}$), 8.34 (1H, d, J = 2, $H\text{-}6_{Py}$).

Mass spectrum (*m*/*z*): 533 $[M – H_2O]^+$.

(3*Z*,22*E*,24*R*)-3-(2′-Chloropyridin-5′-ylmethyloximino)-ergosta-4,7,22-trien-9α-ol (10). A solution of selenium dioxide (0.083 g, 0.748 mmol) in dioxane (4 mL) was stirred, treated in one portion with **6** (0.1 g, 0.187 mmol), stirred for 10 min, and filtered through a layer of Al_2O_3. The sorbent was washed successively with dioxane (5 mL) and CH_2Cl_2 (20 mL). The filtrate was evaporated to ~1/3 the initial volume in vacuo. The residue was treated with CH_2Cl_2 (30 mL) and washed successively with aqueous $NaHCO_3$ solution (5%, 2 × 15 mL) and H_2O (4 × 15 mL). The organic phase was dried over $MgSO_4$. The desiccant was filtered off. The filtrate was evaporated in vacuo. The product was isolated by successive crystallizations first from EtOAc and then from a petroleum ether (70–90°C):EtOAc mixture to afford **10** (0.048 g, 0.0871 mmol), yield 46.5%, mp 165–174°C.

PMR spectrum (CD_2Cl_2, δ, ppm, J/Hz): 0.60 (3H, s, 18-Me), 0.83 (3H, d, J = 8, 26/27-Me), 0.84 (3H, d, J = 8, 26/27-Me), 0.92 (3H, d, J = 7, 28-Me), 1.03 (3H, d, J = 6.5, 21-Me), 1.21 (3H, s, 19-Me), 2.72 (1H, ddd, J_1 = 20, J_2 = 4.5, J_3 = 3.5, H-6α), 3.07 (1H, ddd, J_1 = 20, J_2 = 6, J_3 = 2.5, H-6β), 5.02 (2H, s, $–CH_2–O–N=$), 5.16–5.26 (3H, m, H-7, 22, 23), 6.58 (1H, d, J = 2.0, H-4), 7.31 (1H, d, J = 8, H-3_{Py}), 7.67 (1H, dd, J_1 = 8, J_2 = 2.5, H-4_{Py}), 8.35 (1H, d, J = 2.5, H-6_{Py}).

Mass spectrum (*m*/*z*): 533 $[M – H_2O]^+$.

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