STUDIES ON THE CHEMICAL TRANSFORMATION OF ROTENOIDS. II[1]. REACTIONS OF ROTENONE AND ROTENONONE WITH PRIMARY AMINES

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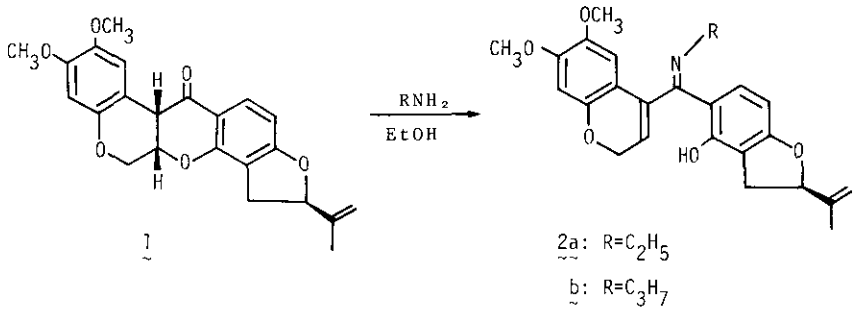
Abstract——The ring transformations of rotenone and rotenononone by primary amines were investigated. The improved synthetic methods for rotenononone were also developed. Rotenone (1) underwent cleavage of C-12a and O-13 linkage to give [1-(4-hydroxy-2-methylethenyl-2,3-dihydrobenzofuran-5-yl)-1-(6,7-dimethoxy-2H-chromen-4-yl)methylidene]alkylamines (2a-b), while rotenononone (5) gave 2-alkylcarbamoyl-4-alkylimino-8-methylethenyl-3-(2-hydroxy-4,5-dimethoxy)phenyl-8,9-dihydro-4H-furo[2,3-h]-[1]benzopyrans (7a-d) as a result of nucleophilic attack on C-12 carbon. Compound 1 was oxidized by nitrosyl chloride or dimethyl sulfoxide in the presence of iodine and sulfuric acid to provide retenononone (5) in improved yields respectively.

(-)-(6aS,12aS,2R)-Rotenone (1) is an abundant natural product and widely used as a useful insecticide. In a previous paper[1], we reported the ring transformation of 1 into 3-substituted 1-(4-hydroxy-2-methylethenyl-2,3-dihydrobenzofuran-5-yl)-7,8-dimethoxy-1,9b,2,3,3a,4-hexahydro-[1]benzopyrano[3,4-c]pyrazol-1-enes by the reaction with monosubstituted hydrazines in the strong basic medium, and discussed on the stereochemistries of the products. In continuation of our study on the chemical transformation of rotenoids and subsequent development of pharmacologically effective compounds, we wish to report in this paper some ring trasformations of rotenone (1) and rotenononone (5) by primary amines.

Treatment of 1 with excess of ethylamine solution in boiling ethanol resulted in cleavage of C-12a and O-13 linkage to provide [1-(4-hydroxy-2-methylethenyl-2,3-dihydrobenzofuran-5-yl)-1-(6,7-dimethoxy-2H-chromen-4-yl)methylidene]ethylamine

(2a) after repeated chromatography on silica gel. This compound was confirmed as the assigned structure 2a on the basis of ^{1}H-NMR spectrum which showed an methine proton of 2H-pyran ring as a triplet centered at δ 5.61 and phenolic hydroxy proton at δ 16.40 respectively. Similarly, 1 was converted to [1-(4-hydroxy-2-methyl-ethenyl-2,3-dihydrobenzofuran-5-yl)-1-(6,7-dimethoxy-2H-chromen-4-yl)methylidene]-propylamine (2b) with propylamine. Compounds 2a-b were fairly unstable, and the reactions of 1 with other primary amines gave no stably isolable compounds.



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2a: R=C_2H_5

b: R=C_3H_7

Chart 1

Table 1. [1-(2,3-Dihydrobenzofuran-5-yl)-1-(2H-chromen-4-yl)methylidene]-alkylamines (2a-b)

Compd.	Yield (%)	mp(°C) (recryst. solvent)	Appearance	Formula	Analysis(%) Calcd(Found) C	H	N
2a	73	52-54 (n-hexane)	yellow amorphous powders	$C_{25}H_{27}NO_5$	71.24 (71.55	6.46 6.64	3.32 3.51)
2b	52	69-74 (n-hexane-Et_2O)	yellow amorphous powders	$C_{26}H_{29}NO_5$	71.70 (71.59	6.71 6.87	3.22 3.50)

Table 2. ^{1}H-NMR and MS Data for 2a-b

Compd.	^{1}H-NMR ($CDCl_3$) δ	MS *m/z*
2a	4.88(2H, d, *J*=4 Hz, C(2)-H_2 of 2*H*-chromene) 5.61(1H, t, *J*=4 Hz, C(3)-H of 2*H*-chromene) 16.40(1H, br s, OH)	421(M^+), 392($M^+-C_2H_5$)
2b	4.90(2H, d, *J*=4 Hz, C(2)-H_2 of 2*H*-chromene) 5.64(1H, t, *J*=4 Hz, C(3)-H of 2*H*-chromene) 16.50(1H, br s, OH)	435(M^+), 392($M^+-C_3H_7$)

Under the same condition, we investigated analogous condensation of rotenonone (5) with primary amines. Compound 5 has been synthesized by a few methods[2], however, these methods were unsatisfactory in terms of chemical yield and reaction time. Therefore, we attempted two kinds of reactions in order to develop the improved synthetic methods for 5 as shown in Chart 2.

Rotenone (1) was suspended in a saturated solution of nitrosyl chloride in dichloromethane and stirred overnight at room temperature to give an inseparable mixture of (2R)-6a,12a-dehydrorotenone (3) and (2R)-12-ethoxy-6a,12a-dehydrorotenone (4). Successive exposure of nitrosyl chloride over a mixture of 3 and 4 completed oxidation to provide 56.5 % yield of rotenonone (5) and 3 % yield of compound 4. As reported by Crombie[3], the formation of 5 was rationalized by the initial oximation at C-12 of 3 followed by the hydrolysis to ketone 5. However, a possible mechanism for the formation of 4 is not clear at present.

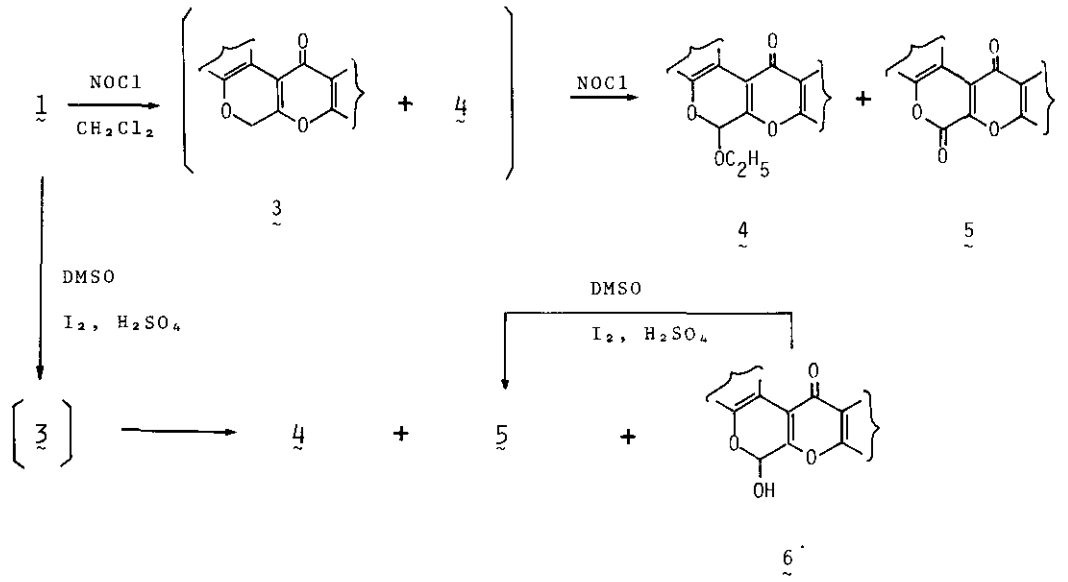


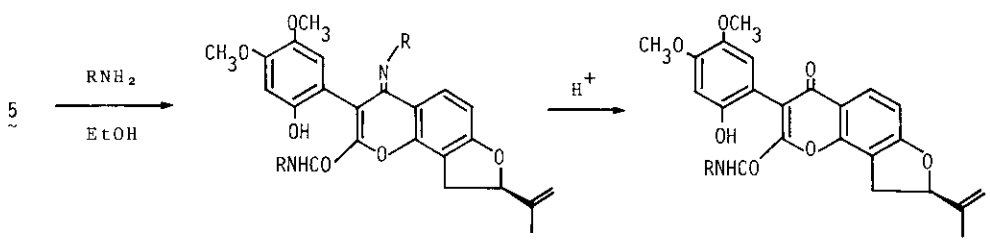
Chart 2

Another attempted oxidation was carried out by a modification of Kornblum oxidation[4], that is, compound 1 was heated under reflux for 15 h with iodine and concentrated sulfuric acid in dimethyl sulfoxide. After separation by column chromatography, 5 was isolated as a major product in 45.8 % yield, accompaning with 4.6 % yield of 4 and 1.9 % yield of (2R)-12-hydroxy-6a,12a-dehydrorotenone (6).

12-Hydroxy compound 6 was readily converted to 5 under the same oxidative condition

and confirmed as the proposed structure 6 because ^{1}H-NMR spectrum has been found to be identical with that of amorpholone 1 isolated by Sorensen and co-workers[5]. Although the isolation of 4 seemed to be strange, we speculated that the formation of 4 was resulted from a reaction between compound 6 and ethoxide ion since it seemed reasonable that rotenone (1) solvated by ethanol during the period of extraction from plants, liberated ethanol into the reaction medium when 1 was converted to 3. This speculation was proved chemically under Kornblum condition as follows; namely, rotenone recrystallized in advance from carbon tetrachloride, has never produced compound 4, while compound 4 has been obtained as a major product in more than 50 % yield when freshly prepared 6a,12a-dehydrorotenone (3)[6] was oxidized in the presence of excess ethanol.

12-Ethoxy compound (4) obtained each time through the oxidative reactions was unknown product and confirmed as the assigned structure 4 on the basis of the following spectral data. ^{1}H-NMR spectrum showed the appearance of new signals attributed to ethoxy and C-12 methine protons, and ^{13}C-NMR spectrum exhibited the existence of ethoxy group at δ 15.21 and δ 64.53. In addition, mass spectrum showed the molecular ion peak at m/z 436 and the base ion peak at m/z 391 (M^+-OC_2H_5). Rotenonone (5) obtained in this manner was then subjected to condensation with ethylamine in boiling ethanol, however, the reaction proceeded in a different manner from rotenone to afford 2-ethylcarbamoyl-4-ethylimino-8-methylethenyl-3-



7a: R=C_2H_5
b: R=$(CH_2)_2CH_3$
c: R=$CH(CH_3)_2$
d: R=$(CH_2)_3$N⟨O⟩

8: R=$CH(CH_3)_2$

Chart 3

Table 3. 2-Alkylcarbamoyl-4-alkylimino-8-methylethenyl-3-(2-hydroxy-4,5-dimethoxy)phenyl-8,9-dihydro-4H-furo[2,3-h][1]benzopyrans (7a-d)

Compd.	Reaction time (h)	mp(°C) (recryst. solvent)	Appearance	Formula	Analysis(%) Calcd(Found) C	H	N	Yield (%)
7a	4	110-114 (n-hexane)	yellow amorphous powders	$C_{27}H_{30}N_2O_6$	67.77 (68.00	6.32 6.45	5.85 5.78)	59
7b	5	163-166 (petr.ether-Et_2O)	yellow amorphous powders	$C_{29}H_{34}N_2O_6$	68.76 (68.93	6.77 6.59	5.53 5.41)	60
7c	8	130-134 (petr.ether-Et_2O)	yellow amorphous powders	$C_{29}H_{34}N_2O_6$	68.76 (68.57	6.77 6.60	5.53 5.39)	33
7d	4	112-114 (petr.ether-Et_2O)	yellow amorphous powders	$C_{37}H_{48}N_4O_8$	65.66 (65.91	7.15 6.98	8.28 8.41)	79

Table 4. ^{1}H-NMR and MS Data for 7a-d

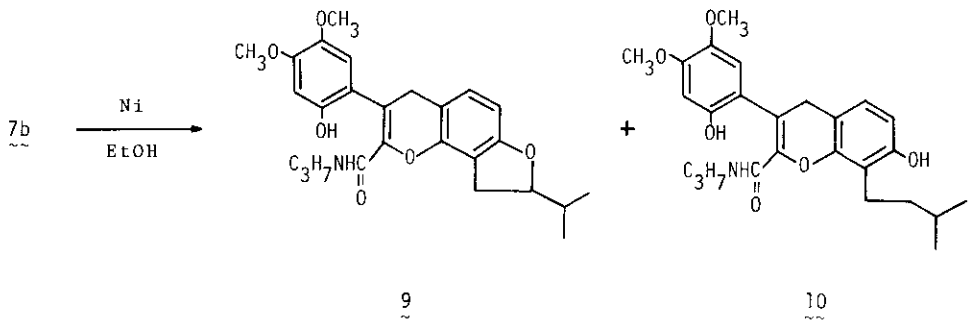
Compd.	^{1}H-NMR($CDCl_3$)δ	MS *m/z*
7a	1.20 and 1.25(6H, t, 2 x CH_3CH_2), 6.46(1H, br s, NH), 16.44(1H, br s, OH)	478(M^+) 406(M^+-$CONHC_2H_5$)
7b	0.96(6H, t, 2 x CH_3CH_2), 1.67(4H, m, 2 x $CH_3CH_2CH_2N$), 3.34(4H, t, 2 x CH_2N), 6.63(1H, br s, NH), 16.57(1H, br s, OH)	506(M^+) 420(M^+-$CONH(CH_2)_2CH_3$)
7c	1.18 and 1.20(12H, d, 2 x $(CH_3)_2CHN$) 3.52 and 4.12(2H, sept, 2 x $(CH_3)_2CHN$), 6.28(1H, br s, NH), 16.64(1H, br s, OH)	506(M^+) 420(M^+-$CONHCH(CH_3)_2$)
7d	8.50(1H, br s, NH), 16.26(1H, br s, OH)	676(M^+) 505(M^+-$CONH(CH_2)_3$ )

(2-hydroxy-4,5-dimethoxy)phenyl-8,9-dihydro-4H-furo[2,3-h][1]benzopyran (7a) as outlined in Chart 3. The spectral data of 7a were sufficient to assign the structure 7a because ^{1}H-NMR spectrum showed phenolic hydroxy proton at δ 16.44, and mass spectrum showed the molecular ion peak at m/z 478 and the base ion peak at m/z 406. Similarly, rotenonone (5) was condensed with other primary amines to

provide corresponding 2-alkylcarbamoyl-4-alkylimino-8-methylethenyl-3-(2-hydroxy-4,5-dimethoxy)phenyl-8,9-dihydro-4H-furo[2,3-h][1]benzopyrans (7b-d). The physical and spectral data of 7a-d were summarized in Table 3 and Table 4.

The chemical proof of the products was made by treating of compound 7c with 5 % hydrochloric acid to provide 2-isopropylcarbamoyl-8-methylethenyl-3-(2-hydroxy-4,5-dimethoxy)phenyl-8,9-dihydro-4H-furo[2,3-h][1]benzopyran-4-one (8) in quantitative yield. Mass spectrum of 8 showed the same type of base ion peak at m/z 379 (M^+-CONHCH$(CH_3)_2$) as seen in that of 7c, and ^{1}H-NMR spectrum showed an amido proton at δ 6.72.

Attempted reductions of compound 7b was then carried out in order to investigate the reactivities against the reducing agents. Only when 7b was hydrogenated with Raney nickel at elevated temperature, the reaction proceeded to give unusual product 9 and 10 in 42.3 and 11.9 % yield respectively as shown in Chart 4.



9 10

Chart 4

On the basis of the spectral data as described in experimental section, these products were confirmed as 8-isopropyl-3-(2-hydroxy-4,5-dimethoxy)phenyl-2-propylcarbamoyl-8,9-dihydro-4H-furo[2,3-h][1]benzopyran (9) and 7-hydroxy-8-isopentyl-3-(2-hydroxy-4,5-dimethoxy)phenyl-2-propylcarbamoyl-3,4-dihydro-2H-chromen-2-ene (10). Compound 9 seemed to be formed by reductive elimination of the benzylic amino group at C-4, while compound 10 was obviously arised from cleavage of dihydrofuran ring of 9.

Compound 2a, 7b and 7c were submitted to Brion research institute of Taiwan in order to test their pharmacological activities. Compound 2a was found to be fairly toxic though it exhibited weak diuretic and antiasthematic activities, while

Compound 7b and 7c were less toxic and exhibited collagen-induced platelet aggregation as well as aspirin.

EXPERIMENTAL

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Mass spectra were measured with a Hitachi M-52 mass spectrometer. ^{1}H-NMR spectra were measured with a Japan Electron Optics Laboratory Co., JNM-100 spectrometer using tetramethylsilane as an internal standard. ^{13}C-NMR spectra were obtained with JEOL Model FX Fourier transform nuclear magnetic resonance spectrometer. Abbreviations are as follows; s, singlet; d, doublet; t, triplet; q, quartet; sept, septet; br, broad. Rotenone (1) was offered by Dr. Kengo Kagei, Eisai Tsukuba Research Laboratory and used without recrystallization.

General Procedure for the Preparation of [1-(4-Hydroxy-2-methylethenyl-2,3-dihydrobenzofuran-5-yl)-1-(6,7-dimethoxy-2H-chromen-4-yl)methylidene]alkylamines (2a-b)

A solution of 1 and 10-15 times molar of amines in 20 ml of EtOH (or $CHCl_3$) was refluxed for 45-60 h under nitrogen until TLC indicated the disappearance of 1. After removal of solvent, the oily residues were chromatographed on silica gel with $CHCl_3$. Analytical, physical and spectral data are sammarized in Table 1 and Table 2.

Rotenonone (5)

a) To a suspension of 20 g of 1 in 60 ml of CH_2Cl_2 was added dropwise a solution of 80 ml of CH_2Cl_2 containing 10 g of NOCl at room temperature during 1 h. After the addition was over, the reaction mixture was then stirred for 24 h and evaporated *in vacuo* below 30°C. On trituration of the residue with Et_2O, 18.3 g of light yellow solids were obtained which were proved to be a mixture of 3 and 12-ethoxy-6a,12a-dehydrorotenone (4) by ^{1}H-NMR spectrum. Since the Rf values of two products were very close each other, the mixture of two products was used for the subsequent oxidation without isolation. A solution of 18.3 g of the mixture in 60 ml of CH_2Cl_2 was treated with 13 g of NOCl in 90 ml of CH_2Cl_2 for 23 h at room temperature, evaporated *in vacuo* and chromatographed on silica gel with $CHCl_3$ as an eluate. Elution of fast moving band afforded (2R)-12-ethoxy-6a,12a-dehydrorotenone (4). Recrystallization from n-hexane-Me_2CO gave 0.66 g (3 %) of light yellow needles, melted at 201-202°C. Anal. Calcd for $C_{25}H_{24}O_7$: C, 68.80; H, 5.54. Found: C, 68.71; H, 5.36. IR(KBr)cm^{-1}: 1645(C=O). ^{1}H-NMR($CDCl_3$)δ: 1.26(3H, t, J=8 Hz, CH_3CH_2O), 3.82(2H, q, J=8 Hz, CH_3CH_2O), 5.89(1H, s, C(12)-H).

^{13}C-NMR($CDCl_3$) δ: 15.21(q, $\underline{C}H_3CH_2O$), 64.53(t, $CH_3\underline{C}H_2O$), 94.49(d, $\underline{C}H(OC_2H_5)$). MS m/z: 436(M^+), 391(M^+-OC_2H_5). Elution of slow moving band afforded rotenonone (5). Recrystallization from EtOH-$CHCl_3$ gave 12.5 g (56.5 %) of fine yellow needles, melted at 298-300°C. Spectral data were identical in all respects with authentic 5[7].

b) A mixture of 5 g (12.7 mM) of 1, 478 mg (1.88 mM) of I_2, 0.1 ml (1.88 mM) of c.H_2SO_4 in 40 ml of DMSO was heated at 140°C for 15 h and poured into ice-water. The resulting precipitates were filtered, dissolved in $CHCl_3$ and chromatographed on silica gel. From the fast moving band, 0.25 g (4.6 %) of 4 and 2.36 g (45.8 %) of 5 were isolated successively. The slow moving band gave 0.1 g (1.9 %) of (2R)-12-hydroxy-6a,12a-dehydrorotenone (6). Recrystallization from $CHCl_3$ gave fine yellow needles, melted at 227-230°C (decomp.)(lit.[5] 255-260°C(decomp.)).

General Procedure for the Preparation of 2-Alkylcarbamoyl-4-alkylimino-8-methylethenyl-3-(2-hydroxy-4,5-dimethoxy)phenyl-8,9-dihydro-4H-furo[2,3-h][1]benzopyrans (7a-d).

A suspension of 5 (5.1 mM) and primary amines (10 mM) in 10 ml of EtOH was refluxed under nitrogen for 4-8 h until reactants have dissolved completely. After removal of solvent, the residual oils were chromatographed on silica gel using $CHCl_3$ as an eluate. Analytical, physical and spectral data are summarized in Table 3 and Table 4.

2-Isopropylcarbamoyl-8-methylethenyl-3-(2-hydroxy-4,5-dimethoxy)phenyl-8,9-dihydro-4H-furo[2,3-h][1]benzopyran-4-one (8)

A solution of 0.1 g of 7c and 10 ml of 5 % HCl was heated at 80°C for 16 h and evaporated in vacuo. The residue was washed with water and recrystallized from n-hexane-$CHCl_3$ to give colorless prisms, melted at 110-111°C. Yield 1.03 g (95 %). Anal. Calcd for $C_{26}H_{27}NO_7$: C, 67.09; H, 5.85; N, 3.01. Found: C, 67.31; H, 5.69; N, 3.25. IR(KBr)cm^{-1}: 1660(C=O). ^{1}H-NMR($CDCl_3$) δ: 1.24(6H, d, $(C\underline{H}_3)_2CHNH$), 4.16 (1H, sept, $(CH_3)_2C\underline{H}NH$), 6.72(1H, br s, NH), 12.36(1H, s, OH). MS m/z: 465(M^+), 379(M^+-$CONHCH(CH_3)_2$).

Catalytic Hydrogenation of 7b

A solution of 0.28 g of 7b in 80 ml of EtOH was hydrogenated over 0.6 g of Raney nickel at 80°C under the pressure of 100 kg/cm^2. After 20 h, the catalyst was removed by suction and the filtrate was evaporated in vacuo to give light yellow powders which were chromatographed on silica gel with $CHCl_3$ as an eluate. From

the fast moving band, the major and more polar product was obtained as colorless prisms (0.053 g, 42.3 %), and this product was confirmed as 8-isopropyl-3-(2-hydroxy-4,5-dimethoxy)phenyl-2-propylcarbamoyl-8,9-dihydro-4H-furo[2,3-h][1]benzopyran (9), mp 221-224°C(EtOH). Anal. Calcd for $C_{26}H_{31}NO_6$: C, 68.86; H, 6.89; N, 3.09. Found: C, 69.06; H, 6.98; N, 3.15. ^{1}H-NMR($CDCl_3$) δ: 1.0(6H, d, $(CH_3)_2CH$), 4.24(2H, s, C(4)-H_2), 4.45(1H, m, C(8)-H), 6.80(1H, br s, NH), 9.65(1H, s, OH). MS m/z: 453(M^+), 367(M^+-$CONHC_3H_7$). The minor and slow moving product was obtained as fine colorless needles (0.015 g, 11.9 %) and confirmed as 7-hydroxy-8-isopentyl-3-(2-hydroxy-4,5-dimethoxy)phenyl-2-propylcarbamoyl-3,4-dihydro-2H-chromen-2-ene (10), mp 190-191°C(n-hexane-$CHCl_3$). Anal. Calcd for $C_{26}H_{33}NO_6$: C, 68.55; H, 7.30; N, 3.07. Found: C, 68.32; H, 7.49; N, 3.25. ^{1}H-NMR($CDCl_3$) δ: 0.36-1.80(5H, m, 2 x CH_2 and $(CH_3)_2CH$), 0.92(6H, d, J=7 Hz, $(CH_3)_2CH$), 2.60(2H, t, $CH_2CH_2CH(CH_3)_2$), 4.26(2H, s, C(4)-H_2), 6.72(1H, s, NH), 6.80(1H, br s, C(7)-OH), 9.20(1H, s, OH of 4,5-dimethoxyphenyl). MS m/z: 455(M^+), 369(M^+-$CONHCH_2CH_2CH_3$).

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