Formation of Pyrido[3,2-*f*]quinoxalines by Reaction of 6-Amino-2,3-dimethylquinoxaline with Aldehydes

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The reaction of 6-amino-2,3-dimethylquinoxaline (1) with acetaldehyde in ethanol proceeded at refluxing temperature to give 10-ethoxy-2,3,8-trimethyl-7,8,9,10-tetrahydropyrido[3,2-*f*]quinoxaline (2), which was converted to 2,3,8-trimethylpyrido[3,2-*f*]quinoxaline (4) and 2,3,8-trimethyl-7,8,9,10-tetrahydropyrido[3,2-*f*]quinoxaline (5) in an acidic medium. Crotonaldehyde also reacted smoothly at room temperature with 1 to afford several pyrido[3,2-*f*]quinoxaline derivatives along with 4 and 5.

Key words pyrido[3,2-*f*]quinoxaline; Skraup reaction; 6-aminoquinoxaline; acetaldehyde; crotonaldehyde

Recently, carcinogenic or mutagenic imidazoquinoxaline derivatives have been isolated from boiled fish and meat, pyrolysates of amino acids and proteins, and from model reaction mixtures consisting of creatine, hexoses and amino acids (Maillard reaction).[1] We have reported the total synthesis of 2-amino-1,7,9-trimethylimidazo-[4,5-*g*]quinoxaline (7,9-DiMeIgQx), which is a new mutagen found in beef extract.[2,3] In the course of our studies, we have found that 6-aminoquinoxalines themselves, regarded as a common structural unit of mutagenic imidazoquinoxalines, showed potent mutagenic activity.[4] These facts make it desirable to examine the presence of 6-aminoquinoxalines in various Maillard reaction mixtures. However, their chemical properties and reactivities have not been clarified. The reaction with aldehydes is of interest because an aldehyde is considered as a key reactive species in Maillard reactions. This paper deals with the formation of pyrido[3,2-*f*]quinoxalines by Skraup reaction of 6-aminoquinoxalines with aldehydes.

We carried out the reaction of 6-amino-2,3-dimethylquinoxaline (**1**), which shows relatively potent mutagenicity.[4] A solution of **1** and acetaldehyde in ethanol was refluxed for 5 h to afford 10-ethoxy-8-methyl-7,8,9,10-tetrahydropyrido[2,3-*f*]quinoxaline (**2**), the structure of which was elucidated on the bases of elemental analysis, FAB-MS and NMR spectra as described in the experimental section. In the ^{1}H-NMR spectrum, the peaks at δ 1.34 (t) and 3.82 (q) can be assigned to an ethoxyl moiety. Three peaks due to methyl groups are observed at δ 1.32 (d), 2.61 (s), and 2.65 (s). The methines and methylene protons could be assigned on the basis of decoupling experiments. It was of interest that the gas chromatogram of **2** showed two peaks. The GC-MS revealed that one peak showed a molecular ion at *m/z* 223 and the other at *m/z* 227, corresponding to the molecules formed by loss of two hydrogens and addition of two hydrogens after elimination of ethanol from **2**, respectively. However, no molecular ion corresponding to **2** was observed. These two compounds were isolated by treatment of **2** in an acidic medium and their structures were determined as 2,3,8-trimethylpyrido[3,2-*f*]quinoxaline (**4**) and 2,3,8-trimethyl-7, 8, 9, 10-tetrahydropyrido[3,2-*f*]quinoxaline (**5**). High-resolution mass spectra (HRMS) of **4** and **5** showed molecular ion peaks at *m/z* 223.1131 (calcd for $C_{14}H_{13}N_3$, 223.1109) and *m/z* 227.1419 (calcd for $C_{14}H_{17}N_3$, 227.1423), respectively. In the ^{1}H-NMR spectra, **4** shows the signals of 4 aromatic protons, 3 singlets due to methyl groups and a broad peak of NH. On the other hand, **5** shows 2 singlets and a doublet due to methyl groups, together with the signals due to two aromatic protons, 5 non-aromatic protons and NH. Their ^{13}C-NMR spectra also support the proposed structures.

This reaction seems to be initiated by addition of the amino group of 2,3-dimethyl-6-aminoquinoxaline (**1**) to crotonaldehyde formed *in situ* from two molar equivalents of acetaldehyde or to the azomethine formed from crotonaldehyde and **1**. Compounds **4** and **5** would be produced by the disproportionation reaction of 2,3,8-trimethyl-7,8-dihydropyrido[3,2-*f*]quinoxaline (**3**) formed by elimination of ethanol in the acidic medium or on heating. It was also demonstrated by the FAB-MS measurement that the ethoxyl group at the 10-position of **2** is easily substituted with a nucleophile. The FAB-MS using *m*-nitrobenzyl alcohol as a matrix showed a relatively strong band at *m/z* 379 due to the molecular ion $[M+H]^+$ of the corresponding 10-*m*-nitrobenzyloxy analog.

The reaction of **1** with 2.2 molar eq of crotonaldehyde proceeded smoothly even at room temperature to give several pyridoquinoxaline derivatives (**6**, **7**, **8**, **9a** and **9b**), along with **4** and **5**. The structures of **6**, **7** and **8** were determined by elemental analyses and comparison of the spectral data with those of **2**, **4** and **5**. In the FAB-MS of **9a** and **9b**, molecular ion peaks $[M+H]^+$ were observed at *m/z* 450 and both molecular formulae were confirmed to be $C_{28}H_{30}N_6$ by elementary analyses. The ^{1}H-NMR spectrum of **9a** shows 4 singlets at δ 1.34, 2.31, 2.69 and 2.71 due to methyl groups bound to aromatic rings, and peaks due to the other methyl groups at δ 1.32 (d) and δ 1.67 (dd). The singlet at δ 1.34 ($ArCH_3$) resonates at extremely high magnetic field comparing with those of the other methyl protons ($ArCH_3$). This suggests that the methyl protons are strongly shielded by placing above an aromatic ring. The protons of an allyl group and 4 methines were assigned as described in the experimental section with the aid of decoupling experiments. The ^{1}H- and ^{13}C-NMR spectra of **9b** were quite similar to those of **9a**. Based on these spectral data we propose the dimeric structure for **9a**, as displayed in Chart 2, and its di-

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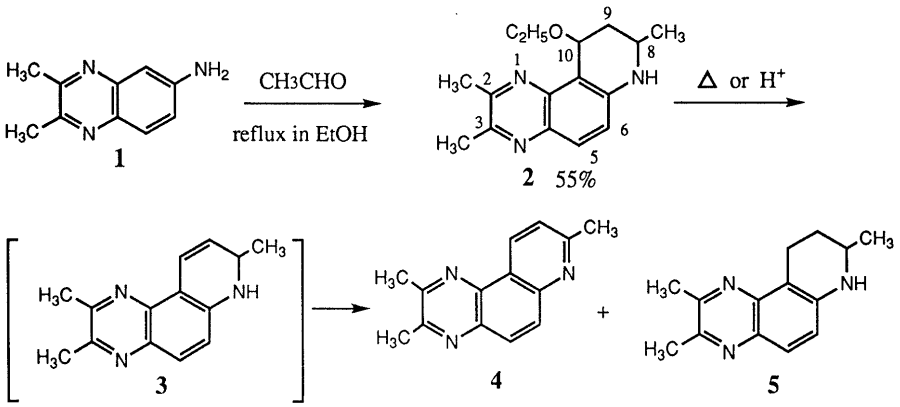

Chart 1. Reaction of 6-Amino-2,3-dimethylquinoxaline with Acetaldehyde

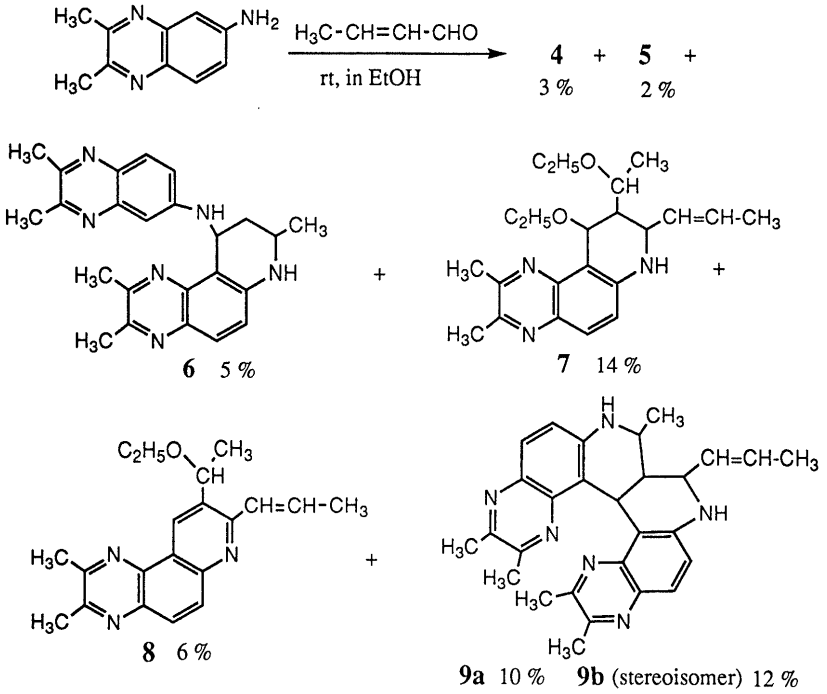

Chart 2. Reaction of 6-Amino-2,3-dimethylquinoxaline with Crotonaldehyde

astereomeric structure for **9b**, although the exact stereochemistry is not clear.

Because of the high reactivity of crotonaldehyde and the strong nucleophilicity at the 5-position of 6-aminoquinoxaline, the reaction of **1** proceeds smoothly, generating many heterocyclic compounds *via* several different intermediates. In the case of the reaction with acetaldehyde, crotonaldehyde is thought to be formed slowly from acetaldehyde under reflux in the presence of a week base catalyst such as **1** in a protic solvent.[5] The crotonaldehyde, once formed, may react immediately with **1** to form **2** under the conditions used. At any rate, it seems unlikely that 6-aminoquinoxalines themselves can be isolated from Maillard reaction mixtures.

Experimental

All melting points were determined on a Yazawa micro melting point apparatus and are uncorrected. Infrared (IR) spectra were measured with a JASCO A-102 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained with a JEOL JNM-GSX270 (^{1}H: 270 MHz, ^{13}C: 67.5 MHz) or JNM-GSX500 (^{1}H: 500 MHz, ^{13}C: 125 MHz) Fourier transform spectrometer using tetramethylsilane (TMS) as an internal standard. Coupling constants (J values) are given in hertz (Hz) and the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High-resolution (HRMS) and low-resolution mass spectra were obtained on a JEOL JMS-AX505w mass spectrometer. FAB-MS (*m*-nitrobenzyl alcohol matrix) were measured with a JEOL JMS-SX102 mass spectrometer.

Reaction of 6-Amino-2,3-dimethylquinoxaline (1) with Acetaldehyde in Ethanol Acetaldehyde (100 mg, 2.2 mmol) was added to a solution of 6-amino-2,3-dimethylquinoxaline (**1**) (173 mg, 1 mmol) in ethanol (5 ml). The solution was refluxed for 5 h with stirring and concentrated under reduced pressure to give an oily residue, which was subjected to PTLC on silica gel (hexane : AcOEt = 1 : 1) to give yellow needles (149 mg, 55%).

2: mp 160 °C (dec.). IR (KBr): 3290 cm^{-1} (NH). FAB-MS *m/z*: 272 $[M+H]^+$. *Anal.* Calcd for $C_{16}H_{21}N_3O$: C, 70.82; H, 7.80; N, 15.48. Found: C, 70.49; H, 7.76; N, 15.45. ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, J=6.9, $C\underline{H}_3CH_2O$), 1.32 (3H, d, J=6.3, $C\underline{H}_3CH$), 1.44 (1H, dt, J=3.0, 13.7, 9-H_A), 2.20 (1H, dt, J=2.4, 13.7, 9-H_B), 2.61 (3H, s, $ArCH_3$), 2.65 (3H, s, $ArCH_3$), 3.72—3.85 (1H, m, 8-H), 3.80 (2H, q, $CH_3C\underline{H}_2O$), 4.25 (1H, br, NH), 5.41 (1H, dd, J=2.4, 3.0, 10-H), 6.90 (1H, d, J=8.8, ArH), 7.64 (1H, d, J=8.8, ArH). ^{13}C-NMR ($CDCl_3$) δ: 15.84, 21.84, 22.33, 23.26, 34.72, 42.22, 63.94, 66.59, 111.46, 120.40, 128.55, 135.35, 141.66, 144.77, 147.46, 152.27.

Reaction of 10-Ethoxy-2,3-8-trimethyl-7,8,9,10-tetrahydropyrido[3,2-*f*]quinoxaline (2) in Acidic Medium Two drops of concentrated hy-

drochloric acid were added to a solution of **2** (271 mg, 1 mmol) in tetrahydrofuran (THF) (10 ml) and the solution was stirred for 1 h at room temperature. After addition of aqueous sodium bicarbonate and removal of THF under reduced pressure, dichloromethane was added to the residue. The organic solution was separated, washed with brine, dried over $MgSO_4$ and concentrated to dryness. A resulting solid was subjected to PTLC (hexane : AcOEt = 2 : 1) to give a colorless powder, 2,3,8-trimethylpyrido[3,2-*f*]quinoxaline (**4**), (85 mg) and yellow crystals, 2,3-8-trimethyl-7,8,9,10-tetrahydropyrido[3,2-*f*]quinoxaline (**5**), (90 mg).

4: needles (hexane), mp 142—143 °C. HRMS (*m/z*): Calcd for $C_{14}H_{13}N_3$: 223.1109. Found: 223.1131. ^{1}H-NMR ($CDCl_3$) δ: 2.79 (3H, s, $ArCH_3$), 2.82 (3H, s, $ArCH_3$), 2.83 (3H, s, $ArCH_3$), 7.52 (1H, d, J=8.4, ArH), 8.10 (1H, d, J=9.4, ArH), 8.15 (1H, d, J=9.4, ArH), 9.35 (1H, d, J=8.4, ArH). ^{13}C-NMR ($CDCl_3$) δ: 22.89, 23.11, 25.27, 120.56, 122.57, 124.03, 129.88, 131.07, 132.52, 138.33, 139.52, 146.86, 152.42, 160.26.

5: prisms (hexane), mp 134—136 °C . HRMS *m/z*: Calcd for $C_{14}H_{17}N_3$: 227.1423. Found: 227.1419. IR (KBr): 3300 cm^{-1} (NH). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, d, J=6.4, $C\underline{H}_3CH$), 1.67 (1H, dddd, J=5.4, 9.8, 10.7, 13.5, 9-H_A), 2.10 (1H, ddt, J=3.2, 6.4, 13.5, 9-H_B), 2.63 (3H, s, $ArCH_3$), 2.66 (3H, s, $ArCH_3$), 3.02 (1H, ddd, J=6.4, 10.7, 17.1, 10-H_A), 3.45 (1H, ddd, J=3.2, 5.4, 17.1, 10-H_B), 3.51 (1H, ddq, J=3.2, 6.4, 9.8, 8-H), 4.10 (1H, br, NH), 6.90 (1H, d, J=8.8, ArH), 7.59 (1H, d, J=8.8, ArH). ^{13}C-NMR ($CDCl_3$) δ: 21.06, 22.25, 23.33, 29.41, 47,12, 112.55, 120.22, 126.64, 135.75, 141.16, 144.24, 147.57, 152.08.

Reaction of 6-Amino-2,3-dimethylquinoxaline (1) with Crotonaldehyde A solution of **1** (1.7 g, 10 mmol) and crotonaldehyde (1.4 g, 20 mmol) in ethanol (60 ml) was stirred for 24 h at room temperature. After removal of the solvent, 7 compounds (**4**; 67 mg, 3%, **5**; 45 mg, 2%, **6**; 100 mg, 5%, **7**; 510 mg, 14%, **8**; 190 mg, 6%, **9a**; 225 mg, 10% and **9b**; 270 mg, 12%) were isolated from the resulting residue by medium-pressure liquid chromatography (Merck, LiChroprep Si 60, AcOEt : hexane = 1 : 1).

6: prisms (hexane–AcOEt), mp 110—111 °C (dec.). IR (KBr): 3300 cm^{-1} (NH). FAB-MS *m/z*: 399 $[M+H]^+$. *Anal.* Calcd for $C_{24}H_{26}N_6$: C, 72.34; H, 6.58; N, 21.09. Found: C, 72.10; H, 6.42; N, 21.48. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, d, J=6.4, $CHC\underline{H}_3$), 1.60 (1H, m, $C\underline{H}_A H_B$), 2.41 (3H, s, $ArCH_3$), 2.45—2.48 (1H, m, $CH_A\underline{H}_B$), 2.56 (3H, s, $ArCH_3$), 2.65 (3H, $ArCH_3$), 2.68 (3H, s, $ArCH_3$), 3.58—3.77 (1H, m, $CH_3C\underline{H}NH$), 4.12 (1H, br, NH), 4.29 (1H, br, NH), 5.62 (1H, m, $CHC\underline{H}NH$), 6.92 (1H, d, J=8.8, ArH), 7.01 (1H, dd, J=2.4, 8.8, ArH), 7.25 (1H, d, J=2.4, ArH), 7.66 (1H, d, J= 9.3, ArH), 7.72 (1H, d, J=9.3, ArH). ^{13}C-NMR ($CDCl_3$) δ: 21.73, 22.42, 22.60, 23.13, 23.35, 34.09, 42.80, 44.79, 104.04, 108.06, 111.22, 120.15, 120.56, 121.35, 128.70, 129.02, 131.21, 135.44, 135.70, 143.39, 144.62, 147.62, 148.06, 152.97.

7: needles (hexane–AcOEt), mp 137—139 °C. IR (KBr): 3280 cm^{-1} (NH). FAB-MS *m/z*: 370 $[M+H]^+$. *Anal.* Calcd for $C_{22}H_{31}N_3O_2$: C, 71.51; H, 8.46; N, 14.08. Found: 71.23; H, 8.20; N, 13.89. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7.1, $C\underline{H}_3CH_2O$), 1.22 (3H, d, J=6.4, $CHC\underline{H}_3$), 1.22 (3H, t, J=7.0, $C\underline{H}_3CH_2O$), 1.63 (3H, d, J=6.4, $=CHC\underline{H}_3$), 2.30 (1H, m, $CHC\underline{H}CH$), 2.61 (3H, s, $ArCH_3$), 2.65 (3H, s, $ArCH_3$), 3.07 (1H, dq, J=6.4, 9.1, O-$C\underline{H}CH_3$), 3.11 (1H, dq, J=7.1, 9.1, $CH_3C\underline{H}_A H_B O$), 3.47 (1H, dq, J=7.1, 9.1, $CH_3CH_A\underline{H}_B O$), 3.76 (1H, dq, J=7.0, 9.4, $CH_3C\underline{H}_A H_B O$), 3.80 (1H, dq, J=7.0, 9.4, $CH_3CH_A\underline{H}_B O$), 3.90 (1H, m, NCH), 4.40 (1H, br, NH), 5.51 (1H, d, J=1.5, $C_2H_5OC\underline{H}$), 5.55 (1H, dq, J=6.4, 15.1, $=C\underline{H}CH_3$), 6.13 (1H, ddq, J=1.4, 6.4, 15.1, $C\underline{H}=CHCH_3$), 6.86 (1H, d, J=9.0, ArH), 7.64 (1H, d, J=9.0, ArH). ^{13}C-NMR ($CDCl_3$) δ: 15.42, 15.62, 17.40, 17.83, 22.45, 23.38, 46.13, 53.19, 63.64, 64.73, 68.24, 75.07, 110.98, 118.60, 120.31, 123.86, 129.02, 134.30, 135.72, 142.81, 147.65, 152.74.

8: needles (AcOEt), mp 130—131°C. FAB-MS *m/z*: 322 $[M+H]^+$. *Anal.* Calcd for $C_{20}H_{23}N_3O$: C, 74.74; H, 7.71; N, 13.07. Found: C, 74.56; H, 7.48: N, 13.00. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, J=6.8, $CH_2C\underline{H}_3$), 1.60 (3H, d, J=6.9, $CHC\underline{H}_3$), 2.06 (3H, dd, J=1.9, 6.8, $=CHC\underline{H}_3$), 2.77 (3H, s, $ArCH_3$), 2.82 (3H, s, $ArCH_3$), 3.48 (2H, m, OCH_2), 4.97 (1H, q, J=6.4, $C\underline{H}CH_3$), 7.03 (1H, dq, J=1.9, 15.2, $C\underline{H}=CHCH_3$), 7.19 (1H, dq, J=6.8, 15.2, $=C\underline{H}CH_3$), 8.04 (1H, d, J=9.2, ArH), 8.14 (1H, d, J=9.2, ArH), 9.41 (1H, s, ArH). ^{13}C-NMR ($CDCl_3$) δ: 15.50, 18.87, 22.86, 23.09, 23.37, 64.40, 74.93, 124.88, 127.27, 129.65, 130.09, 131.32, 134.67, 135.11, 138.44, 139.73, 147.63, 152.30, 152.92, 154.72.

9a: prisms (AcOEt–hexane), mp > 250 °C. IR (KBr): 3300 cm^{-1} (NH). FAB-MS *m/z*: 451 $[M+H]^+$. *Anal.* Calcd for $C_{28}H_{30}N_6$: C, 74.64; H, 6.71; N, 18.65. Found: C, 74.28; H, 6.81; N, 18.54. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, d, J=6.9, $CHC\underline{H}_3$), 1.34 (3H, s, $ArCH_3$), 1.67 (3H, dd, J=1.6, 6.7, $CH=CHC\underline{H}_3$), 2.17—2.19 (1H, m, CH–$C\underline{H}$(–CH)$_2$), 2.31 (3H, s, $ArCH_3$), 2.69 (3H, s, $ArCH_3$), 2.71 (3H, s, $ArCH_3$), 4.15—4.18 (2H, m, NH–$C\underline{H}$–CH–$C\underline{H}$–NH), 4.04 (1H, br, NH), 4.52 (1H, br, NH), 5.52 (1H, ddq, J=1.6, 6.4, 15.1, $C\underline{H}=CHCH_3$), 5.63 (1H, dq, J=6.7, 15.1, $CH=C\underline{H}CH_3$), 5.90 (1H, d, J=4.5, Ar–CH–Ar), 6.82 (1H, d, J=8.7, ArH), 6.92 (1H, d, J=9.1, ArH), 7.54 (1H, d, J=8.7, ArH), 7.61 (1H, d, J=9.1, ArH). ^{13}C-NMR ($CDCl_3$) δ: 17.75, 19.78, 21.13, 22.04, 22.65, 23.53, 33.60, 41.82, 50.75, 53.99, 113.57, 119.72, 120.02, 126.20, 126.89, 127.53, 135.31, 135.74, 135.82, 136.82, 141.75, 141.97, 142.56, 144.95, 146.61, 147.33, 149.84, 151.43.

9b: prisms (AcOEt–hexane), mp > 250 °C. IR (KBr): 3300 cm^{-1} (NH). FAB-MS *m/z*: 451 $[M+H]^+$. *Anal.* Calcd for $C_{28}H_{30}N_6$: C, 74.64; H, 6.71; N, 18.65. Found: C, 74.28; H, 6.85; N, 18.34. ^{1}H-NMR ($CDCl_3$) δ: 1.35 (3H, s, $ArCH_3$), 1.46 (3H, d, J=6.4, $CHC\underline{H}_3$), 1.67 (3H, d, J=6.0, $CH=CHC\underline{H}_3$), 1.81—1.86 (1H, m, CH–$C\underline{H}$(–CH)$_2$), 2.32 (3H, s, $ArCH_3$), 2.62 (3H, s, $ArCH_3$), 2.68 (3H, s, $ArCH_3$), 3.64 (1H, br, NH), 3.69—3.82 (2H, m, NH–$C\underline{H}$–CH–$C\underline{H}$–NH), 4.47 (1H, br, NH), 5.40—5.48 (1H, m, $C\underline{H}=CHCH_3$), 5.67—5.76 (1H, m, $CH=C\underline{H}CH_3$), 5.79 (1H, d, J=4.0, Ar–CH–Ar), 6.84 (1H, d, J=8.7, ArH), 6.89 (1H, d, J=9.1, ArH), 7.54 (1H, d, J=8.7, ArH), 7.65 (1H, d, J=9.2, ArH). ^{13}C-NMR ($CDCl_3$) δ: 17.81, 21.21, 22.01, 22.57, 22.66, 23.47, 24.11, 39.88, 46.40, 55.44, 112.11, 117.56, 119.51, 120.05, 126.39, 126.57, 127.55, 129.72, 133.22, 135.33, 141.24, 142.12, 142.80, 143.57, 146.27, 146.71, 149.92, 151.33.

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