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Ehrlich's Reaction of Furanoeremophilanes

Chiaki Kuroda,* Tadashi Ueshino, and Hajime Nagano[1]

Department of Chemistry, Rikkyo University, Nishi-Ikebukuro, Toshima-ku, Tokyo 171-8501

[1]Department of Chemistry, Ochanomizu University, Otsuka, Bunkyo-ku, Tokyo 112-8610

Received April 7, 2004; E-mail: kuroda@rikkyo.ac.jp

6-Ethoxyfuranoeremophilane and its synthetic analogue, 4-ethoxy-4,5,6,7-tetrahydrobenzofuran, reacted with *p*-dimethylaminobenzaldehyde (Ehrlich's reagent) under acidic conditions in a 2:1 ratio to afford condensation products in 34% and 78% yields, respectively. 6-Hydroxyfuranoeremophilane and 4-hydroxy-4,5,6,7-tetrahydrobenzofuran afforded the same products as in the case of ethoxy derivatives, respectively. The intermediate 1:1 adduct was not obtained from 6-ethoxyfuranoeremophilane, but obtained from the synthetic analogue in low yield. It was deduced that the colored compound in Ehrlich's test is the cationic species of the intermediate 1:1 adduct, and that the concentration of the colored intermediate in the reaction mixture is very low.

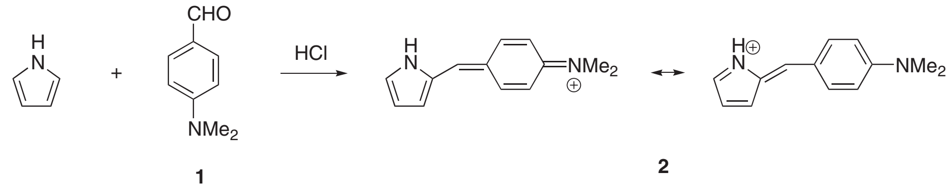
Ehrlich's reagent, *p*-dimethylaminobenzaldehyde (**1**) in hydrochloric acid, has a long history,[1] and is known as the coloring reagent of pyrrole.[2,3] As shown in Scheme 1, a Friedel–Crafts type reaction occurs at the 2- or 3-position of pyrrole to give a colored cation such as **2**. Although this reagent does not color a solution of non-substituted furan, many natural furanosesquiterpenes such as furanoeremophilanes are colored. The pinkish coloring of furanoeremophilanes is probably due to the presence of the electron-rich trisubstituted furan ring. The coloring of furanosesquiterpenes on TLC using this reaction is called Ehrlich's test, which is a very useful method for searching for natural furanosesquiterpenes since it is easy to detect the presence or absence of a furano-compound without isolation. Using the method, various natural furanoeremophilanes were isolated from *Ligularia* (Compositae)[4] and related genera of the family.[5] However, to our surprise, no detailed study on the reaction of *p*-dimethylaminobenzaldehyde (**1**) with natural furanosesquiterpenes has been reported in spite of its usefulness in natural product chemistry for over 40 years. For example, researchers simply stated "compound I was positive to the Ehrlich test" without quoting any literature.[4b,c,5a] Here we report a study on Ehrlich's reaction of natural furanoeremophilanes as well as their synthetic analogues.

Results and Discussion

Two furanoeremophilanes, 6-ethoxy- (**3**)[6] and 6-hydroxyfuranoeremophilanes (= petasalbin, **4**),[4d,7] were used in this study. These compounds were isolated from the rhizome of the cultivated plant *Petasites japonicus* var. *giganteus* (Compositae),[8] which was collected in Nagano Prefecture, Japan. Compound **3** is an artifact derived from **4** during the extraction process with ethanol since this compound was not obtained from the benzene extract.

When an ethanol solution of **3** was treated with *p*-dimethylaminobenzaldehyde (**1**) and HCl at room temperature, the solution immediately changed to pinkish purple and then slowly changed to dark blue. After 1 day of stirring, the reaction was quenched by the addition of aqueous alkali, and the product was separated by silica-gel column chromatography. Although many products were detected on TLC, only the major product could be isolated, and was identified as the 2:1 adduct **5** (34% yield) from its spectral data. Related syntheses of 2:1 adducts from 2-methylfuran and various aldehydes have been reported,[9] however, a 2:1 adduct is not obtained from pyrrole.[3] When **4** was treated under the same reaction conditions, compound **5** was afforded in 21% yield instead of **6** (Scheme 2). This must be the result of substitution of the 6-OH group with solvent ethanol, as in the case of the ethanol extraction process.

From these results, it can be deduced that the pinkish colored compound is the cationic species **7** (three mesomeric structures **7a**, **7b**, and **7c**, are shown in Scheme 3), i.e., the product obtained by the Friedel–Crafts reaction of **3** with *p*-dimethylaminobenzaldehyde (**1**) followed by elimination of the resulting hydroxy group. The 2:1 adduct **5** is considered to be formed from **7** via the attack of a second molecule of **3**. It is known that the pinkish color produced by Ehrlich's test of natural furanoeremophilanes on TLC fades slowly within one hour. This phenomenon can be explained by the formation



Scheme 1.

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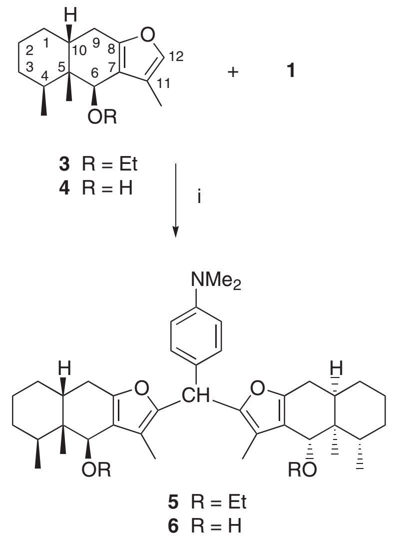
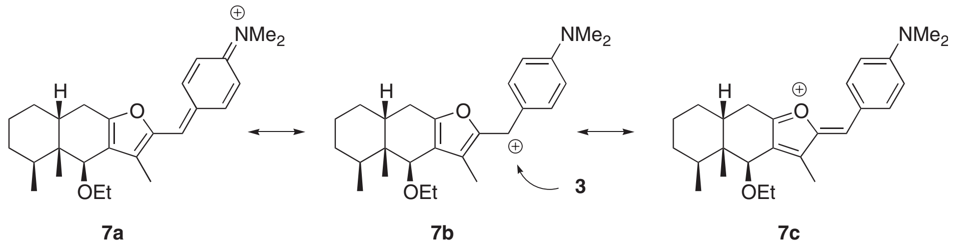


Scheme 2. Reagents and conditions: i) HCl aq, EtOH, r.t., 1 d, then $NaHCO_3$ aq.

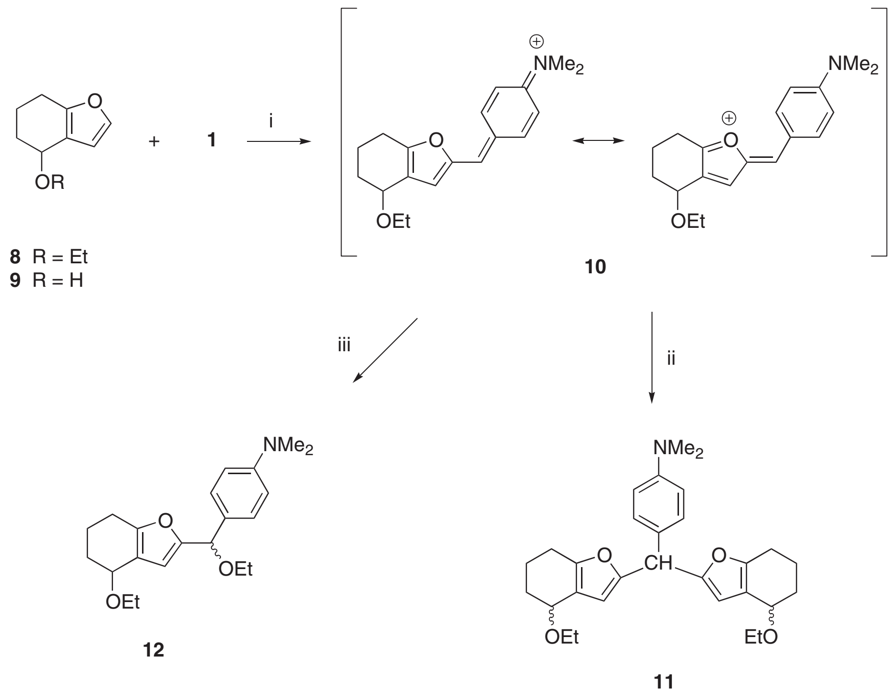
of the colorless compound **5** or other decomposed compounds from **7**. The isolation of the 1:1 adduct was tried by quenching the reaction with aqueous alkali in a short reaction time, however, only the 2:1 adduct **5** was detected together with the substrate **3**. This indicates that the concentration of the colored compound **7** in the flask (thus on TLC in the Ehrlich's test) is low, and to which a second molecule of furanoeremophilane reacted quickly. Namely, the attack of the electron-rich furan ring in **3** to the cationic species **7b** (e.g., the reaction from **7** to **5**) is considered to be faster than that to the protonated *p*-dimethylaminobenzaldehyde (**1**) (e.g., from **3** to **7**).

The Ehrlich's reaction of synthetic analogues **8** and **9** (racemic) was also studied (Scheme 4). The substrate **9** was prepared by the Feist–Benary method from 1,3-cyclohexanedione and chloroacetaldehyde[10] followed by reduction with $LiAlH_4$. Compound **8** was obtained by etherification of **9**. Namely, when **9** was treated with acidic ethanol, **8** was afforded, as in the case of the above Ehrlich reaction.

The results of Ehrlich's reaction of **8** was almost parallel with that of the natural furanoeremophilane **3**, except that the 2:1 adduct **11** was afforded in good yield (78%) after a



Scheme 3.



Scheme 4. Reagents and conditions: i) HCl aq, EtOH, r.t. ii) $NaHCO_3$ aq. after 2 d. iii) $NaHCO_3$ aq. after 1 h.

48 h reaction. In contrast to the reaction of the natural product, of course, **11** was obtained as a mixture of diastereomers. Compound **9** also afforded **11** in 64% yield by the same treatment. The coloring reaction, **8** (or **9**) to **10**, was slower than in the case of natural furanoeremophilanes, since compounds **8** and **9** have only a disubstituted furan ring. The presence of a more reactive trisubstituted furan ring in natural furanoeremophilanes is probably the cause of the complex reaction for **3**. This reactivity difference based on the substitution also explains the difference in yields of **5** and **11**.

When the reaction of **9** was quenched with aqueous alkali in a shorter reaction time (1 h), the presence of the 1:1 adduct **12** was detected on TLC, together with the etherified compound **8**. Compound **12** was obtained in only 1% yield after column chromatography, however, it was difficult to obtain **12** in pure form, and the structure was deduced to be the ethoxy derivative from the spectral data.

In conclusion, the primary result of this study is that the Ehrlich's coloring reaction of natural furanoeremophilanes gives a complex product mixture. This is consistent with the known fact that some natural furanoeremophilanes are not stable enough under weak acid conditions and decompose during handling, even in $CDCl_3$. This can be attributed to the presence of the reactive trisubstituted furan ring. Among the above complex mixture of products, the 2:1 adduct (two furanoeremophilanes and one *p*-dimethylaminobenzaldehyde; the second step is not addition but substitution) was obtained as the major product. It could be deduced that the coloring compound is a dehydrated cationic intermediate of the 1:1 adduct.

Experimental

General Procedure. IR spectra were recorded on a Jasco FT/IR-230 spectrometer. Both ^{1}H and ^{13}C NMR spectra were measured on a Jeol GSX-400 (400 MHz for ^{1}H; 100 MHz for ^{13}C) spectrometer in C_6D_6 as the solvent. Chemical shifts were recorded on the δ scale (ppm) with tetramethylsilane as an internal standard. For ^{13}C NMR, the signal of the solvent (C_6D_6 = 128.0) was used as the reference. Both low-resolution mass spectra (MS) and high-resolution mass spectra (HRMS) were obtained on a Jeol SX-102A, CMATE II, JMS-700, or Shimadzu GCMS-QP5050 mass spectrometer with the EI method unless otherwise noted. Analytical TLC was done on precoated TLC plates (Kieselgel 60 F254, layer thickness 0.2 mm). Wakogel C-200 was used for column chromatography.

Natural Furanosesquiterpenes. Compounds **3** and **4** were isolated from *Petasites japonicus* var. *giganteus* (Compositae) collected in Nagano Prefecture, Japan. Roots of *P. japonicus* var. *giganteus* (ca. 200 g) were collected and, without drying, extracted with ethanol at room temperature for several days. After filtration, the aqueous ethanol solution of the extract was concentrated under reduced pressure to afford a mixture of oily residue and aqueous phase. AcOEt was added, and the organic phase was separated and concentrated. The resulting crude extract was chromatographed on silica-gel (20 g) using hexane/AcOEt as an eluent to afford **3** (117 mg) and **4** (253 mg).

Ehrlich's Reaction. Compound **3** (52.3 mg, 0.200 mmol) was dissolved in commercial EtOH (1 cm^{3}), and to this was added *p*-dimethylaminobenzaldehyde (45.0 mg, 0.302 mmol) and 2 mol dm^{-3} HCl aq (2 drops). The reaction mixture became pinkish purple, and slowly changed to dark blue. After being stirred at room temperature for 1 day, aqueous $NaHCO_3$ was added, and the mixture was extracted with Et_2O. The ethereal layer was washed successively with H_2O and brine, and then dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded an oily residue, which was chromatographed on silica gel (3 g) using hexane/Et_2O (98:2) as the eluent. The chromatography was repeated using hexane/Et_2O (99:1 to 95:5) until **5** (22.3 mg, 34%) was obtained.

Compound 5. An oil; IR (neat) 1614 (C=C), 1520, 1444, 1082, and 737 cm^{-1}; ^{1}H NMR δ 0.63–1.72 (34H, m), 2.05 (3H, s, Me on furan), 2.07 (3H, s, Me on furan), 2.46 (6H, s, NMe_2), 2.37–2.55 (4H, m CH_2–furan × 2), 3.29–3.40 (2H, m, OC*H*HMe × 2), 3.58–3.68 (2H, m, OCH*H*Me × 2), 4.17 (2H, br s, C*H*OEt × 2), 5.87 (1H, s, (furan)$_2$–CH–Ar), 6.58 (2H, d, J = 8.3 Hz, Ar), and 7.24 (2H, d, J = 8.3 Hz, Ar); ^{13}C NMR δ 9.2, 15.8, 15.9, 17.4, 20.4, 25.7, 26.4, 29.8, 31.1, 34.9, 40.3, 41.4, 41.7, 65.4, 76.1, 113.2, 115.6, 119.0, 119.1, 129.1, 148.6, 148.9, and 149.8; MS (FAB) m/z 655 (M^{+}, 100%), 610 (23), and 394 (57); HRMS (FAB) Found: m/z 655.4559 (M^{+}). Calcd for $C_{43}H_{61}NO_4$: M, 655.4603.

Compound 11. An oil; IR (neat) 1614 (C=C), 1520, 1265, 1084, and 737 cm^{-1}; ^{1}H NMR δ 1.09 (6H, two sets of triplet, each J = 6.9 Hz, OCH_2CH_3 × 2), 1.33–1.54 (4H, m), 1.74–1.98 (4H, m), 2.19–2.47 (4H, m), 2.47, 2.49, 2.50 (6H, three singlets, ratio 1:2:1, NMe_2), 3.25–3.50 (4H, m, OCH_2CH_3 × 2), 4.14–4.20 (2H, m, C*H*OEt × 2), 5.54, 5.56 (1H, two singlets, ratio 1:3, ArC*H*(furan)$_2$), 6.25, 6.27, 6.28, 6.30 (2H, four singlets, ratio 1:1:1:1, furan), 6.50–6.59 (2H, m, Ar), and 7.31–7.39 (2H, m, Ar); ^{13}C NMR for the major isomer δ 15.9, 19.3, 23.4, 29.7, 40.3, 45.3, 63.7, 71.1, 108.4, 113.1, 119.5, 129.6, 150.1, 152.1, and 154.1 (one carbon signal could not be specified because of overlapping with the solvent signal); MS m/z 463 (M^{+}, 10%), 429 (10), 341 (19), 281 (49), and 207 (100); HRMS Found: m/z 463.2677 (M^{+}). Calcd for $C_{29}H_{37}NO_4$: M, 463.2723.

Compound 12. An oil; IR (neat) 1606 (C=C), 1520, 1265, and 739 cm^{-1}; ^{1}H NMR δ 1.13 (3H, t, J = 7.1 Hz, OCH_2CH_3), 1.16 (3H, t, J = 7.0 Hz, OCH_2CH_3), 1.07–1.73 (4H, m), 1.96–2.04 (1H, m), 2.37–2.43 (1H, m), 2.52 (6H, s, NMe_2), 3.30 (1H, dq, J = 9.2, 7.1 Hz, OC*H*HCH$_3$), 3.38 (2H, AB, each q, J = 7.0 Hz, OCH_2CH_3), 3.61 (1H, dq, J = 9.2, 7.1 Hz, OCH*H*CH$_3$), 3.90 (1H, ddd, J = 1.6, 5.5, 11.0 Hz, CH$_2$C*H*OEt), 5.47 (1H, s, ArC*H*OEt), 6.25 (1H, d, J = 1.6 Hz, furan), 6.65 (2H, d, J = 8.8 Hz, Ar), and 7.90 (2H, d, J = 8.8 Hz, Ar); MS m/z 343 (M^{+}, 78%), 314 (8), 298 (100, M^{+} − OEt), 268 (14), 252 (25), and 134 (17); HRMS Found: m/z 343.2101 (M^{+}). Calcd for $C_{21}H_{29}NO_3$: M, 343.2147.

Etherification of 9. Compound **9** (411 mg, 2.98 mmol) was dissolved in EtOH (5 cm^{3}), and conc. HCl (0.25 cm^{3}) was added to this solution at room temperature with stirring. After 4 h, an aqueous solution of NaOH was added to pH 7–8, and the mixture was extracted with Et_2O. Drying over anhydrous $MgSO_4$ followed by evaporation of the solvent gave an oily residue, which was chromatographed on silica gel (15 g) using hexane/Et_2O (98:2) as the eluent to afford **8** (342 mg, 69%).

4-Ethoxy-4,5,6,7-tetrahydrobenzofuran (8). An oil; IR (neat) 1626 (C=C), 1508, 1441, 1086, and 725 cm^{-1}; ^{1}H NMR δ 1.15 (3H, t, J = 6.9 Hz, Me), 1.34–1.57 (2H, m), 1.75–1.99 (2H, m), 2.22–2.46 (2H, m), 3.37 (1H, dq, J = 8.8, 7.0 Hz, OC*H*HCH$_3$), 3.44 (1H, dq, J = 8.8, 7.0 Hz, OCH*H*CH$_3$), 4.18 (1H, t, J = 4 Hz, C*H*OEt), 6.28 (1H, d, J = 1.8 Hz, furan), and 7.08 (1H, d, J = 1.8 Hz, furan); ^{13}C NMR δ 15.9, 19.2, 23.3, 29.7, 63.6, 70.9, 110.7, 118.8, 140.6, and 152.9; MS m/z 166

(M^+, 34%), 138 (75), 121 ($M^+ - OEt$, 100), 109 (80), and 91 (52); HRMS Found: m/z 166.0990 (M^+). Calcd for $C_{10}H_{14}O_2$: M, 166.0994; Anal. Found: C, 72.31; H, 72.26%. Calcd for $C_{10}H_{14}O_2$: C, 8.59; H, 8.49%.

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