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SRS-A Antagonist Pyranoquinolone Alkaloids from East African *Fagara* Plants and their Synthesis

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Abstract—Three pyranoquinolone alkaloids isolated from two East African *Fagara* plants have been found to exhibit SRS-A antagonist action. Their synthesis has been accomplished, using a modified Coppola's method or a thermal cyclization followed by an electrocyclic ring closure. Copyright © 1996 Elsevier Science Ltd

Introduction

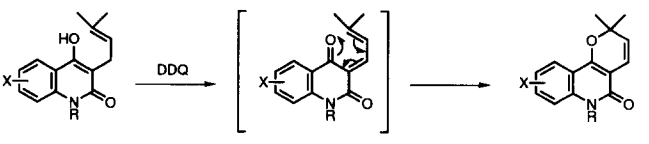
The bark of an East African tree, *Fagara chalybea* Engl. (Rutaceae), is widely used as a folk medicine to cure malaria, colds, coughs, and dizziness. The bark is also chewed to alleviate toothaches. This local medicine is known by different tribal names such as 'cloisuki' by the Masai, 'mdongo' by the Sonjo, and 'mfukambi' by the Shamba. The Masai and Sonjo use this bark as a folk medicine for small children by adding its juice to milk to give the children a better appetite. The decoction is also given to sick goats, especially those suffering from diarrhea.[1] We have recently reported various biological activities of two 2-quinolone alkaloids, flindersine (**1**) and *N*-methylflindersine (**2**), isolated in relatively high concentrations from the *n*-hexane extract of the dried bark of *F. chalybea*.[2,3] The same alkaloids were also isolated from the bark of *F. holtziana*, but found in much lower concentrations. Bioassay guided fractionation led to the isolation of *N*-methylflindersine from the same sources as an insect growth inhibitor against several lepidopterous insects[4,5] in an artificial diet feeding assay.[6] In addition, a related congener 7,8-dimethoxy-*N*-methylflindersine (**3**)[7] was subsequently isolated in minute amount from both *Fagara* barks. The broad antimicrobial activity of these alkaloids[3] may relate to their use for the treatment of diarrhea. Nevertheless, despite their wide use, there has been little study to verify their pharmacological effects. Hence, general pharmacological screening was conducted on these alkaloids.

Results and Discussion

Among their various pharmacological activities found, of the greatest potential interest was the apparent SRS-A antagonist action which was confirmed as low as 1 μg mL^{-1} of **3** in the absence of antihistaminic and antiserotonin properties. In addition, good bronchodilator activity of **3** was confirmed on both perfused guinea pig lungs and isolated tracheal preparations. The latter effect was blocked by propranolol, indicating a β_2-agonist action. Moderate positive inotropic activity of **3** was also confirmed on guinea pig left atria. This activity was also inhibited by propranolol, indicating the presence of a β-agonist action again. The other two alkaloids (**1** and **2**) also exhibited similar activities, but slightly less than those of **3**. This unusual and desirable effect deserves further investigation. In order to obtain substantial amounts needed for further pharmacological study, the syntheses of the two alkaloids (**2** and **3**) were carried out.

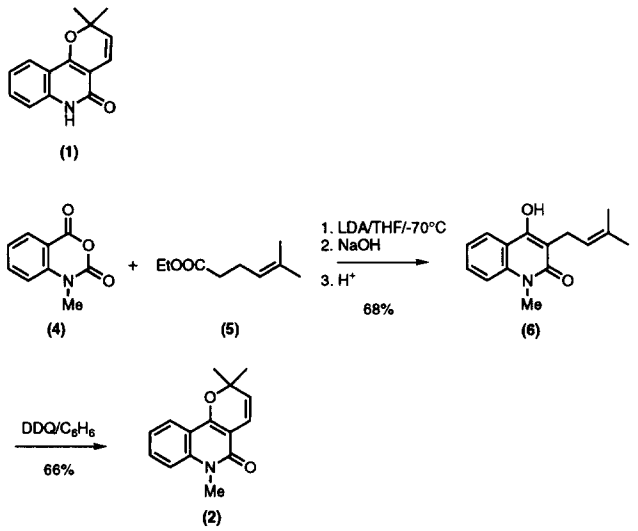
Recently, considerable attention has been given to the synthesis of pyranoquinolone alkaloids.[8,9] Most of the reported syntheses of angular tricyclic alkaloids (III) are based on electrocyclic ring closure of the quinone methide intermediate (II) formed by the oxidation of 4-hydroxy-3-isoprenyl-2-quinolone (I) with dichlorodicyanoquinone (DDQ; Fig. 1). Our syntheses were achieved using similar methodology.

First, *N*-methylflindersine (**2**) was synthesized by Coppola's procedure[10] (Scheme 1). Treatment of the enolate of 5-methyl-4-hexenoate (**5**), which was obtained from 3-methyl-2-butenyl chloride by a malonic ester synthesis followed by decarboxylation, with *N*-methylisatoic anhydride (**4**) gave a 4-hydroxy-



I II III

Figure 1.

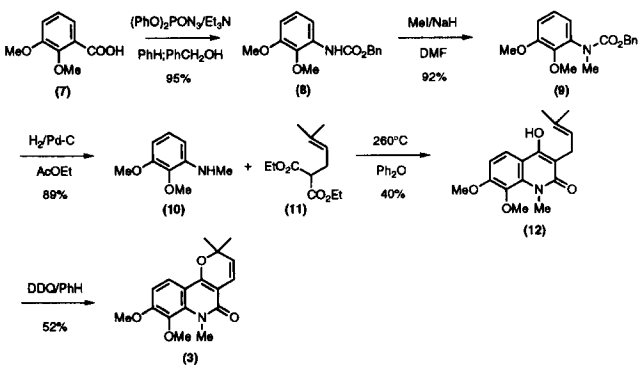


Scheme 1.

3-isoprenyl-2-quinolone (**6**) in 68% yield. Oxidation of **6** with DDQ in boiling benzene yielded a pyranoquinolone (**1**), which was identical with natural *N*-methylflindersine by direct comparison, in 66% yield.

The second target molecule, 7,8-dimethoxy-*N*-methylflindersine (**3**), was synthesized as shown in Scheme 2. The Curtius reaction of 2,3-dimethoxybenzoic acid (**7**) with diphenyl phosphoryl azide[11] followed by heating with benzyl alcohol gave *N*-carbobenzoxy-2,3-dimethoxyaniline (**8**) in 93% yield in one pot. *N*-Methylation of **8** with NaH and CH_3I afforded **9** in 92% yield. Reductive deprotection yielded a *N*-methylamine (**10**) in 89% yield as an oil. Heating **10** with excess diethyl (3-methylbut-2-enyl)malonate (**11**) in diphenyl ether at 230–250 °C yielded 4-hydroxy-2-quinolone (**12**; 39% yield based on **10**, not optimized) along with many other products. The NMR spectrum of **12** showed it to be a mixture of tautomers. Oxidation of **12** with DDQ afforded **3** in 52% yield. Synthetic **2** was identical in all respects with those of the natural alkaloid (mp, IR, UV, and NMR).

In addition to the above mentioned pyranoquinolone alkaloids, a benz[*c*]phenanthrine alkaloid, dihydrochelerythrine, was isolated in large quantities from the



Scheme 2.

same *Fagara* sources.[2] Moreover, various alkaloids have also been isolated from three Brazilian Rutaceae plants.[12–14] None of them exhibited the same SRS-A antagonist activity, indicating specific structural requirements to have this activity. This was supported by the fact that a series of synthetic 8-(benzoylamino)-2-tetrazol-5-yl-1,4-benzodioxans and 8-(benzoylamino)-2-tetrazol-5-yl-4-oxo-4*H*-1-benzopyrans were reported to be potent SRS-A antagonists in both in vitro and in vivo assays.[15] These synthetic compound structures overlap the pyranoquinolone alkaloids (**1**–**3**). Understanding the structural basis for their activity may provide a more rational approach for drug design.

Experimental

General procedure

All bps and mps were uncorrected. All reactions were monitored by TLC carried out on 0.25 mm E. Merck Si gel plates (69F-254). UV light or 7% phosphomolybdic acid in EtOH followed by heating was used as the developing agent. E. Merck Si gels (60, particle size 0.040–0.063 mm) were used for column chromatography. IR spectra were recorded on a Nippon Bunko A-100 IR spectrometer. High-resolution EIMS spectra were obtained on a JEOL LMS-HX 100 spectrometer. 60 and 270 MHz ^1H NMR spectra were recorded on Hitachi R-600L and JEOL GSX-270 spectrometers, respectively.

Chemicals

The pyranoquinolone alkaloids (**1**–**3**) used for the assays were from our previous work.[2–4] THF, C_6H_6, and *n*-hexane were distilled from sodium benzophenone ketyl. CH_2Cl_2 was distilled from P_2O_5. Triethylamine, tetramethylethylenediamine, and DMF were distilled from CaH_2.

Pharmacological assays

General pharmacological assays were performed by Panlabs (Taipei, Taiwan). SRS-A antagonist activity of **3** was confirmed against LTD_4 with an IC_{50} of 20 μM.

4-Hydroxy-(3-methyl-2-butenyl)-1-methyl-2-quinolone (6). To a solution of LDA (2 mmol) in 3 mL of THF was added a solution of ethyl 5-methyl-4-hexenoate (0.090 g, 0.58 mmol) in THF (1.5 mL) at −70 °C. After 1 h of stirring at the same temperature, the resulting enolate was treated with a solution of *N*-methylisatoic anhydride (0.104 g, 0.59 mmol) in 3 mL of THF according to the method of Coppola.[10] After 30 min of stirring, the mixture was quenched with dil hydrochloric acid and extracted with CH_2Cl_2. The combined extracts were dried ($MgSO_4$) and evaporated. The residue was dissolved in methanolic sodium hydroxide solution (2 N, 1 mL) and allowed to stand for 40 min. The mixture was acidified with dil hydrochloric acid and extracted with CH_2Cl_2. The combined extracts were dried ($MgSO_4$) and evapor-

ated. Column chromatography of the residue using Si gel and elution with $CHCl_3$ yielded **6** as colorless needles (95 mg, 68%): mp 165–168 °C (from C_6H_6:*n*-hexane); IR (Nujol): 3250, 1640, 1610, 1565, 1165, and 750 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz): δ 1.80 (d, J=1.6 Hz, 3H), 1.85 (s, 3H), 3.56 (d, J=7 Hz, 2H), 3.73 (s, 1H), 5.42 (brt, J=7 Hz, 1H), 7.24 (ddd, J=1.5, 7.4, 8.0 Hz, 1H), 7.35 (d, J=9 Hz, 1H), 7.56 (ddd, J=2.7, 4.9 Hz, 1H), and 7.96 (dd, J=2, 8 Hz, 1H).

***N*-Methylflindersine (2).** A mixture of **6** (0.1 g, 0.41 mmol) and DDQ (0.115 g, 0.50 mmol) in C_6H_6 (30 mL) was heated under reflux for 2 h. The filtered mixture was evaporated and the residue was taken up in $CHCl_3$. The extract was washed successively with aqueous $NaHCO_3$ solution and brine, dried ($MgSO_4$), and evaporated. Recrystallization of the residue from Et_2O:*n*-hexane gave **2** as pale yellow needles (65 mg, 66%): mp 85.3–86.5 °C. The IR, NMR and MS data were identical with those of the natural product.

***N*-Carbobenzoxy-2,3-dimethoxyaniline (8).** A mixture of diphenyl phosphoryl azide (5.63 g, 0.0205 mol), 2,3-dimethoxybenzoic acid (3.64 g, 0.020 mol) and triethylamine (2.78 mL) in 40 mL of C_6H_6 was heated under reflux for 1.25 h. After the evolution of N_2 had ceased, a solution of benzyl alcohol (2.16 g, 0.02 mol) in 5 mL of C_6H_6 was added and the mixture was heated at reflux for 2 h. After cooling, the mixture was washed successively with cold 1 N HCl, aq $NaHCO_3$ solution, and brine and dried ($MgSO_4$). Evaporation of the solvent yielded **8** (5.36 g, 95%), which was used for the next step without further purification.

***N*-Carbobenzoxy-2,3-dimethoxy-*N*-methylaniline (9).** A mixture of NaH (0.893 g, 0.0223 mol, 60% dispersion in oil, washed twice with *n*-hexane) and **8** (5.36 g, 0.0186 mol) in 12 mL of DMF was stirred at room temperature for 30 min. Then a solution of CH_3I (5.79 mL, 0.093 mol) in 6 mL of DMF was added and the resulting mixture was stirred at room temperature for 1 h. Ice water was then added to this mixture and the resulting mixture was extracted with $CHCl_3$. The combined extracts were washed with brine, dried ($MgSO_4$), and evaporated. The crystalline residue was recrystallized from $CHCl_3$ and *n*-hexane to give **9** as colorless prisms (5.13 g, 92%): mp 92–93 °C; IR (neat): 1710, 1590, 1270, 1160, 1070, 1010 and 745 cm^{-1}; ^1H NMR ($CDCl_3$, 60 MHz): δ 3.19 (s, 3H), 3.72 (s, 3H), 3.84 (s, 3H), 5.13 (brs, 2H), 6.55–7.05 (m, 3H) and 7.27 (m, 5H). Anal. calcd for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65. Found: C, 67.64; H 6.35; N, 4.64.

***N*-Methyl-2,3-dimethoxyaniline (10).** A mixture of **9** (4.43 g, 0.0147 mol) and 10% Pd–C catalyst (880 mg) in EtOAc (50 mL) was hydrogenated under a hydrogen atmosphere. The suspension was filtered and the filtrate was evaporated. The residue was dissolved in $CHCl_3$ and the solution was extracted with dil HCl. The combined aqueous extracts were then made basic with concd NH_4OH, and this aqueous mixture was again extracted with $CHCl_3$. The combined extracts were dried ($MgSO_4$) and evaporated to give **10** as an oil (2.206 g, 89%), which was used for the next step without further purification: IR (neat) 3400, 1600, 1510, 1260, 1220, 1000, 770 and 730 cm^{-1}.

4-Hydroxy-1-methyl-3-(3-methyl-2-butenyl)-7,8-dimethoxy-2-quinolone (12). A mixture of **10** (1.760 g, 0.0105 mol) and diethyl (3-methylbut-2-enyl)malonate (**11**) (4.14 g, 0.0181 mol) in 8 mL of diphenyl ether was heated at 230–255 °C for 2.75 h. The solvent was removed under reduced pressure and the residue was dissolved in $CHCl_3$. The organic layer was washed successively with dil HCl and brine, dried ($MgSO_4$), and evaporated to leave a crystalline residue. The latter was recrystallized from Et_2O:*n*-hexane to afford **12** (1.24 g, 39% yield, based on **10**): mp 155–157 °C (from C_6H_6:*n*-hexane); IR (Nujol): 3100, 1640, 1270, 1230, 1180, 1075, 780 and 770 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz): δ 1.82 (*d*, J=1.3 Hz, 3H), 1.85 (d, J=1.1 Hz, 3H), 3.49 (m, J=1.2, 7.4 Hz, 2H), 3.78 (s, 3H), 3.94 (s, 3H), 3.96 (s, 3H), 5.41 (m, J=1.6, 7.4 Hz, 1H), 6.87 (d, J=9.3 Hz, 1H), and 7.67 (d, J=9.3 Hz, 1H); Anal. calcd for $C_{17}H_{21}NO_4$: C, 67.31; H, 6.98; N, 4.62. Found: C, 67.53; H 6.67; N, 4.63.

7,8-Dimethoxy-*N*-methylflindersine (3). A mixture of **12** (0.188 g, 0.62 mmol) and DDQ (0.195 g, 90%, 0.775 mmol) in 20 mL of C_6H_6 was heated under reflux for 2 h. The filtered mixture was concentrated under vacuum. The residue was taken up in $CHCl_3$ and the extract was washed successively with aq NaOH, dil HCl and dried ($MgSO_4$), and evaporated The residues was purified by flash chromatography (SiO_2, elution with $CHCl_3$:MeOH, 98.5:1.5) to give **3** (97 mg, 52%): mp 85–87 °C (from Et_2O:*n*-hexane). The IR, NMR, UV, and MS data were identical with those of the natural product. Anal. calcd for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.35; N, 4.65. Found: C, 67.59; H 6.39; N, 4.65.

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