Merrekentrones A−D, Ipomeamarone-like Furanosesquiterpenes from *Merremia kentrocaulos*[1]

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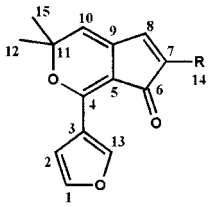
Four new furanosesquiterpenes, merrekentrones A (**1**), B (**2**), C (**3**), and D (**4**), were isolated from the roots and rootstocks of *Merremia kentrocaulos*. Their structures were elucidated on the basis of spectroscopic data interpretation. In contrast to ipomeamarone (**5**), the well-known phytoalexin of *Ipomoea batatas*, **1**−**4** seem to be normal plant constituents. Merrekentrone A (**1**) was also detected in the roots of *M. guerrichii* and *M. aurea*.

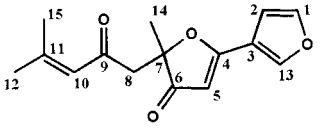
The Convolvulaceae, a large family with about 2000 species distributed mostly in tropical and subtropical parts of the world, is known to possess a wide variety of alkaloids, e.g., ergolines and tropanes.[2−4] Kaurane derivatives have been obtained from *Operculina aurea* (Kellogg) House.[5] Some species contain pentacyclic triterpenes,[6−8] and the isolation of dammarane type triterpenes from *Argyreia capitata* (Vahl) Choisy has been reported.[9] Furanosesquiterpenes such as ipomeamarone (**5**) have been isolated as phytoalexins produced by tubers of sweet potato (*Ipomoea batatas* (L.) Lam.) infected by pathogenic fungi.[10]

During our ongoing research on secondary metabolites from the Convolvulaceae, we investigated *Merremia kentrocaulos* (C.B. Clarke) Rendle, a large woody climber forming a tuberous rootstock occurring in tropical Africa and India. From the roots and rootstocks of this species grown in a greenhouse we isolated four novel sesquiterpenes named merrekentrones A (**1**), B (**2**), C (**3**), and D (**4**). The current report describes the isolation and structure elucidation of these natural compounds.



1 R = CH_3

2 R = CH_2OH



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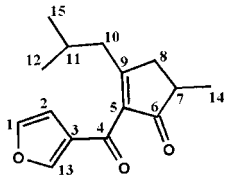

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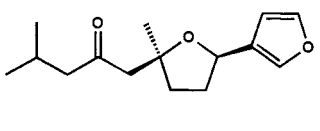

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The dried roots and rootstocks of *M. kentrocaulos* were extracted with MeOH at room temperature. The crude MeOH extract was partitioned between H_2O and CH_2Cl_2. The oily residue of the organic layer was further fractionated by a combination of silica gel column chromatography and preparative HPLC to give sesquiterpenes **1**−**4**.

Merrekentrones A and B (**1**, **2**) showed analogous UV spectra with characteristic absorption bands at λ_{max} 235, 300, and 370 nm, indicating that the compounds possessed extended conjugated π electron systems. The EIMS of **1** exhibited a molecular ion peak at *m*/*z* 242, corresponding to a molecular formula of $C_{15}H_{14}O_3$ (HREIMS). The ^{13}C NMR spectrum showed characteristic signals for a β-substituted furan ring at δ 142.5, 109.2, 120.6, and 147.3. From the $^1H-^{13}C$ COSY NMR spectrum the corresponding proton signals at δ 7.44, 7.19, and 8.97 (1 H, br s, each) could be assigned, revealing an unusual downfield shift of H-2 and H-13.[11] Regarding the molecular formula, besides the furan ring, the structure had to contain two more rings, three double bonds, and one keto group. The ^1H NMR spectrum showed two methyl groups (δ 1.51, 6 H, s) attached to the same carbon, which was pointed out by the correlation to each other in the HMBC spectrum. A further correlation could be observed to a signal at δ 79.8 (C-11), which also was correlated to an olefinic proton at δ 5.53 (H-10). Another olefinic signal at δ 6.98 (H-8) showed a long-range coupling to a ketone at δ 190.4 (C-6), whereas the olefinic methyl group at δ 1.98 exhibited cross-peaks to C-6 and C-8. Thus, a substructure containing an α,β-unsaturated ketone substituted with a methyl group in α-position could be established. The fact that H-8 coupled long-range with three further olefinic carbons at δ 104.6 (C-5), 146.8 (C-9), and 118.0 (C-10), respectively, revealed a conjugation to two double bonds and thus a branching next to this proton. The ^{13}C NMR chemical shifts of the tetrasubstituted double bond between C-4 and C-5 at δ 178.6 and 104.6, respectively, are characteristic for an enolether, establishing the structure of **1**. The downfield chemical shift of the furan protons (H-2, H-13) may be due to the anisotropic effect of the C-6 carbonyl group.

A molecular ion peak at *m*/*z* 258 in the EIMS of **2** corresponded to a molecular formula of $C_{15}H_{14}O_4$. The ^1H NMR data were quite similar to those of **1** with the exception of the methyl groups. Instead of the olefinic methyl group, an oxygen-substituted methylene group was observed, which corresponded to a carbon at δ 58.7 in the ^{13}C NMR spectrum.

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The UV spectrum of **3** differed significantly from those of **1** and **2** and showed an absorption maximum at λ_{max} 298 nm. The EIMS showed a molecular ion peak at *m/z* 260, corresponding to a molecular formula of $C_{15}H_{16}O_4$ (HREIMS). The ^{13}C NMR spectrum again displayed signals for a β-substituted furan ring at δ 143.4 (C-1 and C-13), 116.6 (C-3), and 107.5 (C-2). Furthermore, two isolated olefinic protons, one methylene group, and three methyl groups were observed in the ^1H NMR spectrum. In contrast to **1**, not just one but two of the methyl groups were shifted downfield and thus were attached to a double bond. The methylene protons displayed an AB system at δ 2.97 (d, J = 15.0 Hz) and 2.94 (d, J = 15.0 Hz). The ^{13}C NMR spectrum showed additional signals for a tetrasubstituted carbon at δ 86.9 (C-7) and two carbonyl signals at δ 194.0 (C-6) and 204.5 (C-9). The ^{13}C NMR data of C-5 and C-4 at δ 98.8 and 177.5, respectively, again indicated an enolether moiety. Thus, the chemical structure of **3** was determined as shown.

Compound **4** displayed a molecular ion peak at *m/z* 246, corresponding to a molecular formula of $C_{15}H_{18}O_3$ (HREIMS). Its UV spectrum showed an absorption maximum at λ_{max} 293 nm. The ^{13}C NMR spectrum revealed again the occurrence of a β-substituted furan ring. Furthermore, two keto groups, one tetra-substituted double bond, two methylene groups, two methine groups, and three methyl groups were observed. Two of the methyl groups, displaying doublets at δ 0.90 and 0.92 (J = 6.5 Hz, each), had to be attached to the same carbon, which was supported by the correlation to each other in the HMBC spectrum. The methine proton at C-11 was further coupled to a methylene group at δ 2.46 (H-10) which showed long-range correlations to a further methylene group at δ 39.4 (C-8) and to the two carbon atoms of the tetrasubstituted double bond at δ 141.1 (C-5) and 181.5 (C-9), respectively. Therefore, there had to be a branching between the two methylene groups. The methylene protons of C-8 coupled to a methine proton at δ 2.56 (H-7), which was further coupled to another methyl group (H-14). This methyl group showed HMBC correlations to the carbonyl group at δ 207.6 (C-6). These data allowed us to establish the structure of merrekentrone D (**4**) as shown. The fact that the potentially chiral compounds **3** and **4** displayed an optical rotation of 0° might be explained by enolization in the case of **4** or by attack of the hydroxyl function from different sides to C-7 in the precursor of **3**, thus leading to racemic substances.

The merrekentrones are structurally related to ipomeamarone (**5**). However, ipomeamarone and its derivatives known from *I. batatas* could not be detected in *M. kentrocaulos*. Instead, **4** can be regarded as 6-oxomyomontanone, a derivative of (+)-myomontanone, a hepatotoxic sesquiterpene isolated from *Myoporum montanum* R. Br. (Myoporaceae).[12] This is the first time that ipomeamarone-like sesquiterpenes could be detected and isolated beyond *I. batatas* in the Convolvulaceae. As the merrekentrones were obtained from healthy plants, there is no indication that they are phytoalexins as ipomeamarone and its derivatives in *I. batatas*.

Merrekentrone A (**1**) was also detected by HPLC in roots of the herb *M. guerrichii* Meeuse from Namibia and by GC−MS in roots of *M. aurea* (Kell.) O'Donell, an endemic plant from Baja California. However, merrekentrones are absent in the large Middle American climber *M. tuberosa* (L.) Rendle, as well as in the herbaceous climbers *M. gemella* (Burm. f.) Hallier f. ssp. *gemella* and *M. vitifolia* (Burm. f.) Hallier f. occurring in Southeast Asia.

Experimental Section

General Experimental Procedures. ^1H, ^{13}C, $^1H-^1H$ COSY, and $^1H-^{13}C$ COSY spectra were run in $CDCl_3$ solution on a Bruker AVANCE DPX 400 (400 MHz, TMS as internal standard). HMBC spectra were obtained on a Bruker DRX 500 MHz spectrometer. EIMS were recorded on a Finnigan MAT CH7A (70 eV); HRMS, on a Finnigan MAT 711 (80 eV). Optical rotations were measured with a Perkin-Elmer 241 MC. UV and IR spectra were obtained on a Shimadzu UV-160A and a Perkin-Elmer 1420 spectrophotometer, respectively. Preparative column chromatography was performed on silica gel 60 (70−230 mesh, Merck). Preparative high-performance liquid chromatography (HPLC) separation was performed on a Knauer pumping system with a Knauer variable-wavelength detector (225 nm) equipped with a Knauer Nucleosil 300 C-18 column (10 μm, 22 × 250 mm).

Plant Material. The seeds of *M. kentrocaulos* were collected at the road Masvingo-Mutare in Zimbabwe. The plants were grown in a greenhouse at the Institut für Pharmazie, Berlin, and a herbarium specimen (JS 21) is deposited there.

Extraction and Isolation. The dried roots and rootstocks (400 g) were ground and extracted four times with 1 L MeOH for 4 h at room temperature. The MeOH extract was concentrated under reduced pressure. The viscous concentrate was suspended in 800 mL of 2% aqueous tartaric acid and extracted three times with CH_2Cl_2 (800 mL each). Column chromatography of the CH_2Cl_2 extract (4.6 g) on silica gel and elution with a stepwise gradient of cyclohexane−EtOAc (9:1, 8:2, 7:3, 1:1, 3:7) and finally with EtOAc alone (300 mL each) gave 10 (I−X) fractions. Fraction I, eluting with 9:1 cyclohexane−EtOAc, contained pure **1** (40 mg). Fraction II, eluting with 8:2 cyclohexane−EtOAc, was subjected to preparative HPLC (H_2O−MeOH, 50:50 → 20:80 in 1 h) to afford **4** (5 mg). Fraction V, eluting with 7:3 cyclohexane−EtOAc, was further separated by preparative HPLC with H_2O−MeOH mixtures (55:45 → 20:80 in 1 h) to give **2** (20 mg) and **3** (4 mg).

Merrekentrone A (1): oil; UV λ_{max} (log ϵ) 235 (3.9), 300 (3.6), 370 (3.3) nm; IR (KBr) ν_{max} 3131, 2976, 2929, 2855, 1696, 1621, 1562, 1537, 1503, 1159, 872 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.97 (1H, brs, H-13), 7.44 (1H, brs, H-1), 7.19 (1H, brs, H-2), 6.98 (1H, s, H-8), 5.53 (1H, s, H-10), 1.98 (3H, s, H-14), 1.51 (6H, s, H-12, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 190.4 (s, C-6), 178.6 (s, C-4), 147.3 (d, C-13), 146.8 (s, C-9), 142.5 (d, C-1), 136.3 (d, C-8), 132.2 (s, C-7), 120.6 (s, C-3), 118.0 (d, C-10), 109.2 (d, C-2), 104.6 (s, C-5), 79.8 (s, C-11), 29.2 (q, C-12, C-15), 11.3 (q, C-14); EIMS *m/z* 242 [M$^+$] (32), 227 (100), 213 (4), 199 (11), 95 (15); HREIMS *m/z* 242.0942 (calcd for $C_{15}H_{14}O_3$, 242.0943).

Merrekentrone B (2): oil; UV λ_{max} (log ϵ) 235 (3.9), 301 (3.6), 370 (3.3) nm; IR (film) ν_{max} 3427, 3157, 2976, 2928, 1677, 1654, 1589, 1505, 1156, 874 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.95 (1H, brs, H-13), 7.45 (1H, brs, H-1), 7.17 (1H, brs, H-2), 7.10 (1H, s, H-8), 5.67 (1H, s, H-10), 4.57 (2H, s, H-14), 1.53 (6 H, s, H-12, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.7 (s, C-6), 189.9 (s, C-4), 147.5 (d, C-13), 146.8 (s, C-9), 142.7 (d, C-1), 135.4 (d, C-8), 132.0 (s, C-7), 120.5 (s, C-3), 120.4 (d, C-10), 109.0 (d, C-2), 104.8 (s, C-5), 80.3 (s, C-11), 58.7 (t, C-14), 28.9 (q, C-12, C-15); EIMS *m/z* 258 [M$^+$] (58), 243 (84), 215 (42), 95 (66); HREIMS *m/z* 258.0869 (calcd for $C_{15}H_{14}O_4$, 258.0892).

Merrekentrone C (3): oil; $[\alpha]^{20}_D$ 0° (*c* 0.2, $CHCl_3$); UV λ_{max} (log ϵ) 298 (3.5) nm; IR (KBr) ν_{max} 2978, 2933, 1708, 1650, 1590, 1505, 1158, 873 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.97 (1H, brs, H-13), 7.50 (1H, brs, H-1), 6.67 (1H, brs, H-2), 6.03 (1H, s, H-10), 5.77 (1H, s, H-5), 2.97 (1H, d, J = 15.0 Hz, H-8a), 2.94 (1H, d, J = 15.0 Hz, H-8b), 2.07 (3H, s, H-15), 1.86 (3H, s, H-12), 1.47 (3H, s, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.5 (s, C-9), 194.0 (s, C-6), 177.5 (s, C-4), 156.0 (s, C-11), 143.4 (d, C-1, C-13), 122.6 (d, C-10), 116.6 (s, C-3), 107.5 (d, C-2), 98.8

(d, C-5), 86.9 (s, C-7), 48.6 (t, C-8), 26.7 (q, C-12), 21.5 (q, C-14), 19.9 (q, C-15); EIMS *m*/*z* 260 [M^+] (10), 164 (40), 83 (100); HREIMS *m*/*z* 260.1053 (calcd for $C_{15}H_{16}O_4$, 260.1049).

Merrekentrone D (4): yellow solid; $[\alpha]^{20}_D$ 0° (*c* 0.1, $CHCl_3$); UV λ_{max} (log ϵ) 293 (4.2) nm; IR (KBr) ν_{max} 3131, 2958, 2928, 2870, 1702, 1649, 1626, 1510, 1154, 872 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.86 (1H, brs, H-13), 7.42 (1H, brs, H-1), 6.78 (1H, brs, H-2), 2.95 (1H, dd, J = 19.0, 7.0 Hz, H-8a), 2.56 (1H, m, H-7), 2.46 (1H, d, J = 6.5 Hz, H-10), 2.30 (1H, dd, J = 19.0, 2.5 Hz, H-8b), 1.98 (1H, m, H-11), 1.26 (3H, d, J = 7.5 Hz, H-14), 0.92 (3H, d, J = 6.5 Hz, H-15), 0.90 (3H, d, J = 6.5 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 207.6 (s, C-6), 186.4 (s, C-4), 181.5 (s, C-9), 149.8 (d, C-13), 143.9 (d, C-1), 141.1 (s, C-5), 127.9 (s, C-3), 108.9 (d, C-2), 40.9 (d, C-10), 40.7 (d, C-7), 39.4 (t, C-8), 27.6 (d, C-11), 22.7 (q, C-12), 22.6 (q, C-15), 16.6 (q, C-14); EIMS *m*/*z* 246 [M^+] (48), 231 (25), 203 (22), 175 (53), 163 (41), 147 (25), 95 (100); HREIMS *m*/*z* 246.1266 (calcd for $C_{15}H_{18}O_3$, 246.1256).

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References and Notes

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