Photooxygenation of the Helimers of (−)-Isocolchicine: Regio- and Facial Selectivity of the [4 + 2] Cycloaddition with Singlet Oxygen and Surprising Endoperoxide Transformations

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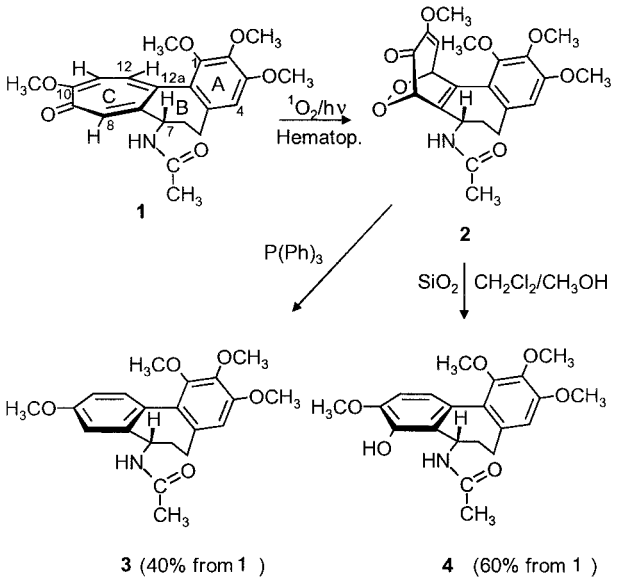
Photooxygenation of the helimeric mixture of (−)-(*M*,7*S*)/(*P*,7*S*)-isocolchicine (**6**) with the superdienophile singlet oxygen has been studied. Cycloaddition occurred with high regioselectivity at the 7a,11-positions of the alkaloid and predominantly at the diene face anti to the amidic substituent at the stereogenic center C-7, leading to two endoperoxides **7** (syn) and **8** (anti) with an 1:7 ratio. The structure of the minor product **7** was established by X-ray analysis. Investigation of the triethylamine induced transformation of the predominant endoperoxide **8** furnished a mixture of two isomers (*M*,7*S*)-**10a**/(*M*,7*S*)-**10b** in a 2:1 ratio possibly with constitutional interconversion and with (*M*,7*S*)-**9** as plausible intermediate. Treatment of this mixture with silica gel/ethyl acetate at ambient temperature surprisingly led to an atropenantiomerically pure colchicinoid (*M*,7*S*)-**12** characterized by an eightmembered oxocine B-ring, the structure and absolute configuration of which could be determined by X-ray analysis. For the unprecedented formation of the novel colchicinoid (*M*,7*S*)-**12** a plausible reaction pathway is suggested, involving a complete transfer of the (*M*) helical asymmetry of the intermediate (*M*)-**11** into (*S*) asymmetry of the newly formed carbon center of (*M*,7*S*)-**12**. Prerequisite for such a scenario is the configurational stability of the intermediate pseudobiaryl (*M*)-**11**, under the conditions employed, allowing to transmit the axial chirality onto the chiral center of the product (*M*,7*S*)-**12**.

Introduction

Recently we have shown that, hitherto unknown, Diels−Alder reactions toward one of the facially dissymmetric diene moieties of the natural alkaloid (−)-(*M*,7*S*)-colchicine (**1**) with several hetero- and carbodienophiles lead to a variety of interesting colchicinoids with novel C-ring alterations.[1] In particular when (−)-colchicine (**1**) was subjected to photooxygenation with singlet oxygen in the presence of hematoporphyrin, an endoperoxide **2** was obtained with high regio- and facial selectivity and in good yield. The colchicinoid **2** proved to be an efficient starting material for convenient syntheses of *N*-acetylcolchinol *O*-methyl ether (**3**) and androbiphenyline (**4**) (Scheme 1),[2] allocolchicinoids, well-known as inhibitors of cell growth and tubulin polymerization, with stronger inhibitory effects than that observed with (−)-colchicine (**1**).[3]

Scheme 1



These findings prompted us to investigate also (−)-isocolchicine (**6**) as a promising novel starting material in our efforts to develop novel colchicinoids with possibly

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more favorable pharmacological profiles compared to the toxic natural (−)-colchicine (**1**). (−)-Isocolchicine (**6**)[3a,b] is an unnatural, semisynthetic constitutional isomer of natural (−)-colchicine (**1**) with a similar skeleton, consisting of the A-, B-, and C-rings. It is easily obtained by *O*-methylation of colchiceine[4] with diazomethane and differs in structure from the naturally occurring alkaloid **1** by the relative positions of the C-ring carbonyl and methoxy moieties and the shifting of double bonds. Recent findings concerning the structure of (−)-isocolchicine (**6**) confirmed earlier assumptions[5,6] that the colchicinoid skeleton of **6** is helically distorted and thus like that of the parental alkaloid characterized by two chiral elements, a chiral axis of the pseudobiaryl moiety and a chiral center at C-7. (−)-Isocolchicine (**6**) has been found to exist as two atropisomeric conformers (*M*,7*S*)-**6** and (*P*,7*S*)-**6** in apolar solvents (Scheme 2).[4,5] Despite the fact that (−)-isocolchicine (**6**) is a bridged pseudobiaryl, the two helimers (*M*,7*S*)-**6** and (*P*,7*S*)-**6** are configuratively stable at least at room temperature, because they are obviously separated by a moderately high barrier of 99.2 kJ·mol^{-1}.[4] Up to now it is unclear why (−)-isocolchicine (**6**) may be found in two atropisomeric forms in solution and (−)-colchicine (**1**) not.[6−8]

The C-rings of both atropisomeric conformers (*M*,7*S*)-**6** and (*P*,7*S*)-**6** are functionalized by two facially differentiated 1,3-diene moieties, the 11,12a- and the 12a,8-diene systems, possibly suitable for hitherto unknown [4 + 2] cycloaddition reactions of (−)-isocolchicine (**6**). Because the steric demand and the electronic nature of the two diene moieties in the tropolone ring C of the two skeletal conformations of (−)-isocolchicine are different, the employed dienophiles on the one side should prefer the most reactive diene of both helimers. On the other side each of the diene moieties possesses two different faces, i.e., syn or anti to the NH-group at the stereogenic center C-7 (Scheme 2). Thus, besides the expected chemoselectivity toward one of the different two diene moieties of (*M*,7*S*)-**6** or (*P*,7*S*)-**6**, π-facial diastereoselectivity should be an important stereochemical feature of (−)-isocolchicine cycloaddition reactions.

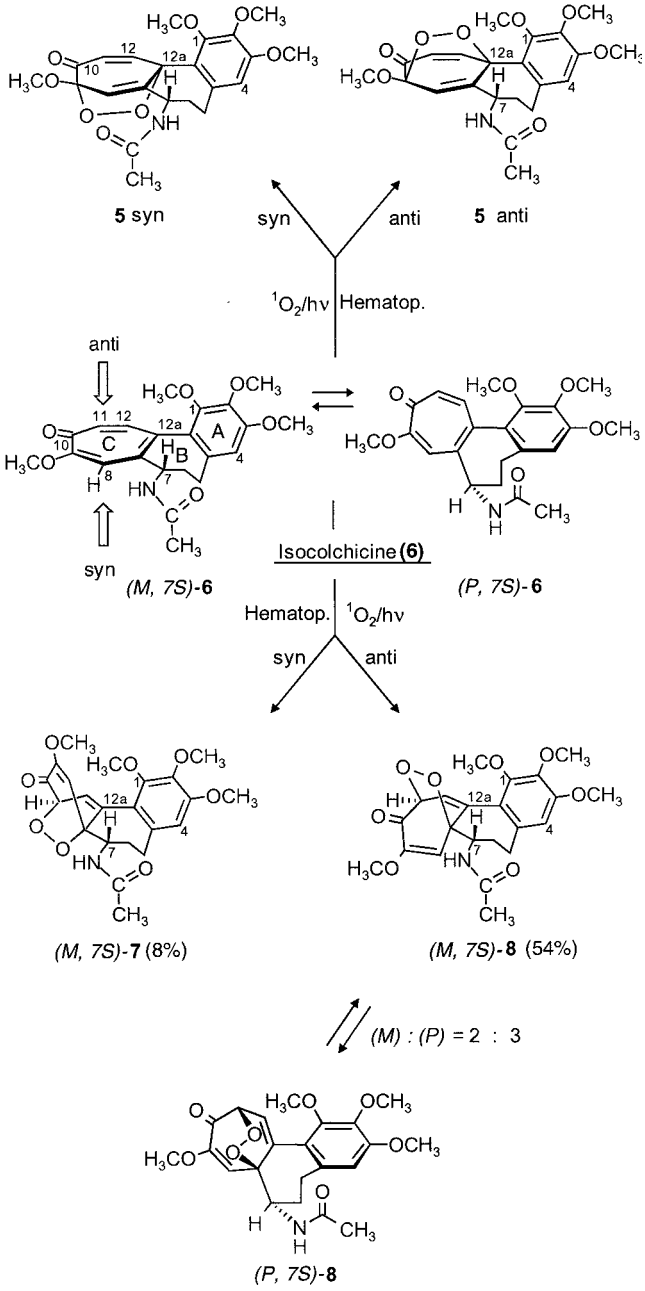
Accordingly we were interested to investigate [4 + 2] cycloadditions with the two helimers of (−)-isocolchicine (**6**) first in order to gain novel colchicinoids and second to study the degree and sense of the chemoselectivity [(*M*)-**6**/(*P*)-**6**], regioselectivity (11,12a- or 12a,8-diene moiety) and π-facial selectivity (syn or anti) of this intriguing helically distorted pseudobiaryl.

We report herein our results on hitherto unkown [4 + 2] cycloadditions reactions of (−)-isocolchicine (**6**) with the superdienophile singlet oxygen[9] and some of the different unexpected successive reactions of the predominant endoperoxide produced.

Results and Discussion

When the helimeric mixture (*M*)-**6**/(*P*)-**6** of (−)-isocolchicine was subjected to photooxygenation in the nonpolar, aprotic solvent $CHCl_3$ (0−20 °C, 30 min) with singlet oxygen $^1O_2$ in the presence of hematoporphyrin,[2] two crystalline endoperoxides **7** and **8** were obtained with 62% total yield in a 1:7 ratio, which were readily separable by careful column chromatography. Endoperoxides of type **5**, syn or anti, could not be isolated, although their formation could not entirely be excluded.

Scheme 2



Detailed spectroscopic analysis (mass, ^{1}H, ^{13}C NMR) indicated that the cycloaddition behavior of (−)-isocolchicine (**6**) was surprisingly different to that observed for (−)-colchicine (**1**).[1,2] It proceeded under the same reaction conditions[2] and with comparably high regioselectivity but at different positions; while (−)-colchicine (**1**) is selectively attacked at the 8,12a-diene moiety, with (−)-isocolchicine (**6**), cycloaddition at the 7a,11-positions prevails. Whereas cycloaddition of $^1O_2$ toward **1** occurred exclusively at the diene face syn to the *N*-acetyl group, the π-facial diastereoselectivity of **6** is lower, and in contrast to **1** anti approach is predominant with **6**.

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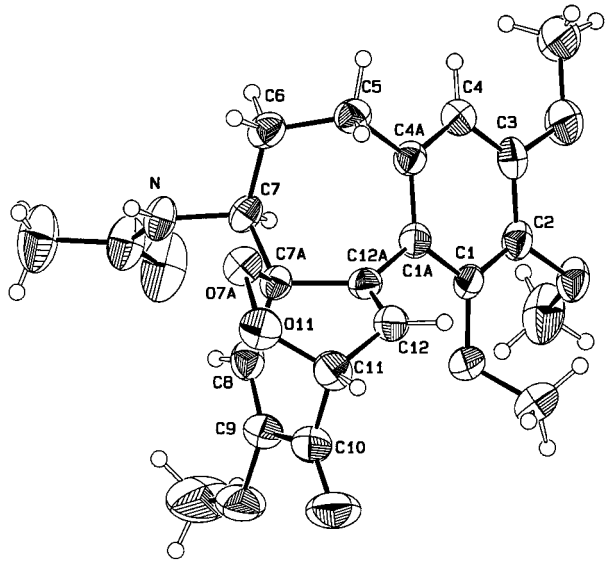

Figure 1. ZORTEP[10] plot of the structure of compound (*M*,7*S*)-**7** determined by X-ray crystallography (thermal ellipsoids at the 50% probability level) showing the crystallographic atom-numbering scheme and solid-state conformation of the (*M*,7*S*)-enantiomer; small open circles represent hydrogen atoms.

In the case of the minor product **7** (yield 8%) appropriate crystals for a X-ray crystallographic analysis were obtained, which conclusively established its constitution and configuration. Figure 1 shows the molecular structure and the atomic numbering used. As can be seen from the ZORTEP[10] drawing of a molecule of **7**, the endoperoxide isolated in minor yield is the result of a regioselective attack of singlet oxygen at the 11,12a-diene moiety of the alkaloid. Furthermore cycloaddition of $^1O_2$ in this case occurred at the diene face syn to the amidic substituent at the stereogenic center C-7. As expected the A ring and the bicyclic C ring are tilted, with respect to the pivot bond (C1A−C12A) as a result of the strain of the molecule, thus giving rise to a (*M*)-helical conformation with the C-7−NH-acetyl group in the energetically favored pseudoequatorial position. The antiparallel orientation of the three methoxy groups is common in crystals of colchicinoids and favored by ca. 4.2 kJ·mol^{-1} compared with the sometimes also-observed parallel orientation of the 1,2-dimethoxy groups of several colchicinoids.[3b,7]

The structure of the major reaction product (54% yield) could be unambiguously elucidated by mass, ^{1}H, and ^{13}C NMR spectral analysis (see Experimental Section), indicating that cycloaddition of $^1O_2$ predominantly occurred toward the diene face of **6** anti to the amidic substituent at the stereogenic center C-7. Like (−)-isocolchicine (**6**), the endoperoxide **8** exists as a mixture of two atropodiastereomeric conformations with a (*M*)-**8**:(*P*)-**8** = 2:3 ratio as indicated from the ^{1}H NMR spectrum in $CDCl_3$ consisting of two sets of signals in the above-mentioned ratio. Indicative for the conformational state [(*M*) or (*P*)] of the endoperoxide **8** is, above all, the chemical shift of the CH_3-protons of the *N*-acetyl group;[5,8,11] as a rule the relevant signal of the (*M*)-conformation of a colchicinoid appears at δ ca. 2.00 and that of the (*P*)-form at δ ca. 1.60.[12]

The predominant endoperoxide **8**, obtained in sufficient amounts, was supposed to be a promising starting material in our attempts to obtain novel semisynthetic structural modifications of (−)-colchicine (**1**). Thus, we first tried to investigate the triethylamine-induced transformations of this intriguing tetracyclic compound. Treatment of the endoperoxide **8** with equimolar amounts of triethylamine at 0 °C led to a crystalline product which could be isolated in 89% yield. The ^{1}H NMR spectrum of the compound in $CDCl_3$ was recognized as a series of doubled and partly broadend signals obviously stemming from a mixture of two isomeric hemiketals (*M*,7*S*)-**10a**/(*M*,7*S*)-**10b.** The singlet for the CH_3-protons of the *N*-acetyl group is diagnostic for the (*M*)-conformational state of the hemiketals.

Unfortunately, all attempts to separate the two species by column chromatography failed, possibly because of constitutional interconversion with the ketol (*M*,7*S*)-**9** as the plausible intermediate. Similar interconverting hemiketals have been isolated recently after cleavage of the peroxide linkage of the dihydrocolchicine 8,12-endoperoxide[2] induced by methanol/silica gel. With DMSO-d_6 instead of $CDCl_3$ as solvent, the two hemiketals seemed to be stable compounds showing two distinct sets of ^{1}H NMR signals in a 2:1 ratio.

Concerning the mechanism of their formation, we propose that the triethylamine-induced deprotonation of, e.g., (*M*,7*S*)-**8** (see Scheme 3) in the first step, as indicated, generates as the initial product the intermediate (*M*,7*S*)-**9**, which reversibly undergoes ring closure by two competing pathways to the hemiketals (*M*,7*S*)-**10a** with a 7a,11-oxygen bridge on the one side and (*M*,7*S*)-**10b** with a 7a,10-oxygen bridge on the other side.

Treatment of the mixture (*M*,7*S*)-**10a**/(*M*,7*S*)-**10b** with silica gel/ethyl acetate at ambient temperature surprisingly led to the tricyclic product (*M*,7*S*)-**12** in 48% isolated yield, a novel optically active colchicinoid with an extended eight-membered B-ring. As for (−)-colchicine (**1**), counterclockwise optical rotation was observed for the annulated oxocine, indicating an atropo-enantioselective rearrangement. Since the enantiomeric purity of (*M*,7*S*)-**12** could not be determined with commercially available chiral shift reagents such as Eu(hfc)$_3$ or Pr(hfc)$_3$ as additives to solutions of (*M*,7*S*)-**12** in $CDCl_3$, we utilized the methodology of Salvadori and co-workers,[13] developed for the determination of enantiomeric purity of binaphthyl derivatives by ^{1}H NMR spectroscopy, simply by adding to the solution of the antipodes an excess of quinine in a NMR tube. Indeed, the enantiomeric purity of (*M*,7*S*)-**12** could be determined by ^{1}H NMR spectroscopy, using quinine as chiral solvating agent. The absorptions at δ 8.17 and 4.15 in the ^{1}H NMR spectrum of the colchicinoid *rac*-**12** in the presence of 3 equiv of quinine were clearly split into two signals at δ 8.09 and 8.11 and δ 4.09 and 4.11, respectively. In contrast, the colchicinoid (*M*,7*S*)-**12** under the same conditions gave only one signal at δ 8.09 and 4.09, respectively; a signal

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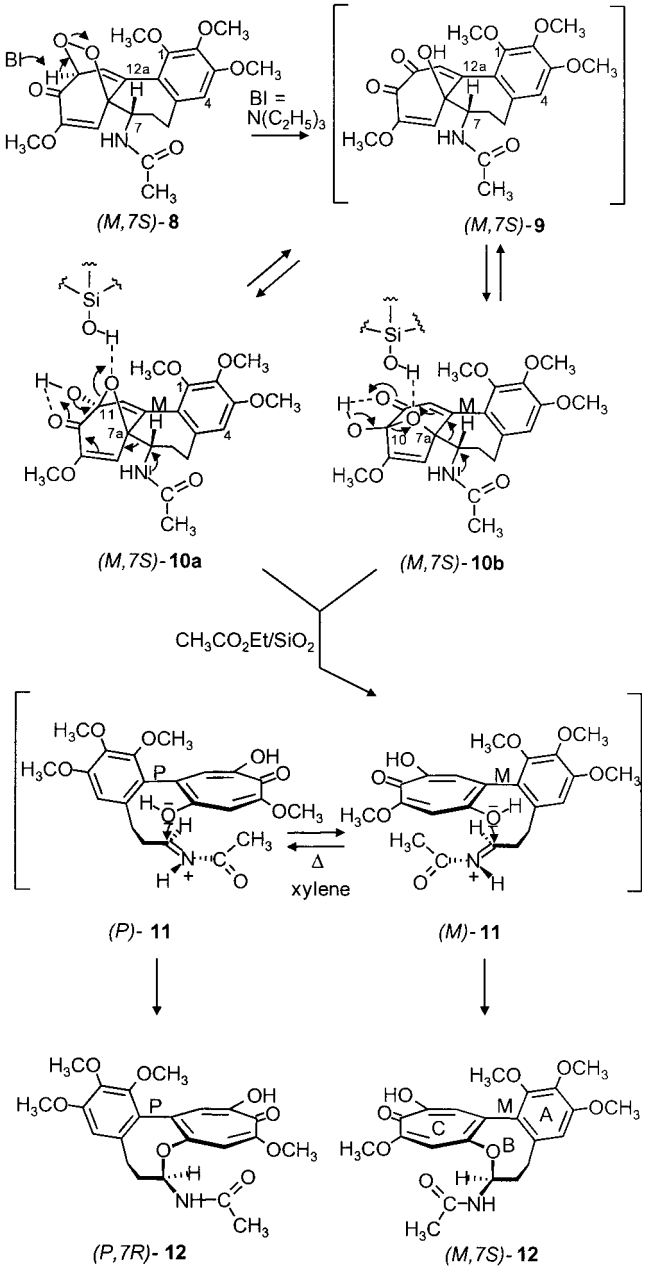
Scheme 3



of the enantiomeric (*P,7R*)-**12** could not be detected. Therefore it was evident that the enantiomeric purity of (*M,7S*)-**12** was >95%. Because spectroscopic data did not allow an unequivocal assignment neither of the constitution nor of the absolute configuration, crystals of this intriguing compound were subjected to X-ray crystallographic analysis. This disclosed that there are three independent molecules of very close geometry in the asymmetric unit and 1.88 partly disordered dichloromethane molecules, thus allowing determination of the absolute configuration at C-7 of the colchicinoid to be 7*S*. Thereby it was indicated that inversion at the stereogenic center had occurred in the course of the rearrangement. A view of the solid-state conformation of this novel colchicinoid with an eight-membered B-ring is shown in Figure 2. The helicity around the pivot bond joining the A and C rings just like in colchicine is (*M*), placing the C-7 acetamido substituent in a pseudoequatorial orientation. The A- and C-rings of all the three independent molecules in the asymmetric unit are fairly planar (max.

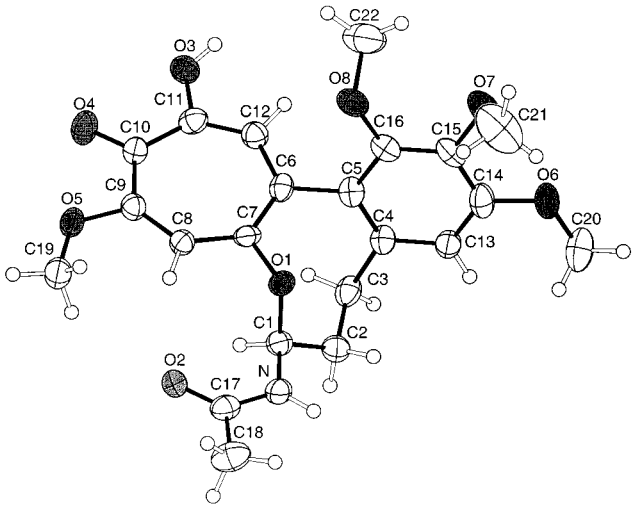


Figure 2. ZORTEP[10] plot of the structure of compound (*M,7S*)-**12** determined by X-ray crystallography (thermal ellipsoids at the 50% probability level) showing the crystallographic atom-numbering scheme and solid-state conformation; small open circles represent hydrogen atoms.

deviation from planarity 0.051(3) Å). The main differences concern the dihedral angles between the least-squares planes of A- and C-rings which are found to be 66.7°, 62.0°, and 57.3°, respectively. This is 12.7°, 8.0°, and 3.3° larger than the average of hitherto known colchicinoid crystals, but significantly smaller in comparison with a recently published colchicine derivative similarly characterized by an eight-membered B-ring lactam exhibiting a dihedral angle of 76.3°.[14]

As to the mechanism concerning the formation of the novel colchicinoid (*M,7S*)-**12**, it seemed to be conceivable that the weakly acidic silica gel induces an atropostereoconservative ring opening, which could result as indicated, from a retro-Mannich fragmentation, giving rise to the formation of an axially chiral pseudobiaryl iminium cation (*M*)-**11**, although the corresponding deprotonated species of (*M*)-**11** could not be excluded as the reactive intermediate. Due to the rather high steric demand of the three substituents ortho to the biaryl axis, it was reasonable to anticipate that this helimer should be configurationally stable at least under the reaction conditions (room temperature) employed. In this connection it is noteworthy to refer to a colchicinoid such as **13** with a similar substitution pattern ortho to the biaryl axis. This is characterized by an at room-temperature stable biaryl axis, which allowed resolution of the racemic species into atropisomeric enantiomers corresponding to a rotational barrier >96 kJ·mol^{-1}.[14] Therefore, no helimerization should occur between the atropo-enantiomeric forms (*M*)-**11** and (*P*)-**11** under the conditions mentioned above. Thus, the chiral information due to the atropisomerism of the pseudobiaryl (*M*)-**11** can be transmitted to the C-7-stereogenity of the tricyclic colchicinoid (*M,7S*)-**12**, obviously one of the rare asymmetric induction processes[15] which involve a complete intramolecular transfer of an axial to a central chiral element.

(*M,7S*)-**12** represents a novel atropenantiomerically pure colchicinoid characterized by an eight-membered oxocine B-ring. Interestingly, further experimental in-

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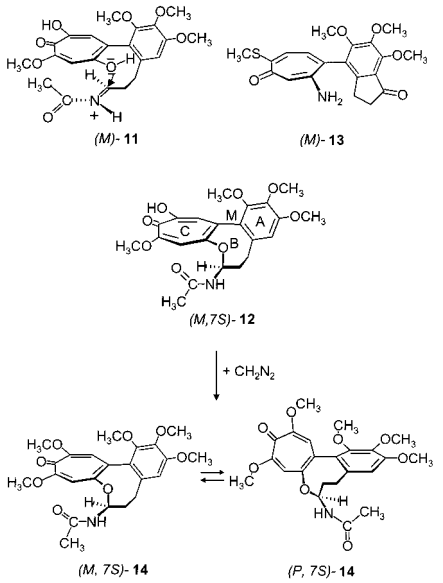
vestigations concerning the rearrangement **10** ⇒ **11** ⇒ **12** revealed that (*M*)-**11** at higher temperatures is obviously configurationally unstable, leading to an interconversion of the two helimeric forms (*M*)-**11**/(*P*)-**11**. Thus, heating of the mixture **10a/10b** in dry xylene at ca. 140 °C results in a complete loss of stereochemical information leading to racemic **12**.

Treatment of the OH-acidic pseudobiaryl (*M*,7*S*)-**12** with an excess of diazomethane led to the chemically stable pentamethoxy-substituted **14** in 54% yield. Detailed analysis of the NMR spectra of **14** revealed that due to the presence of the stereogenic pseudobiaryl axis and the stereocenter at C-7, two sets of ^{1}H NMR data are observed for the two atropo-diastereomeric species (*M*,7*S*)-**14** and (*P*,7*S*)-**14** in a 9:1 ratio. The chemical shifts of the CH_3-protons of the acetamido groups clearly allowed us to distinguish these diastereomers; due to the degree of steric hindrance, the equilibrium is nearly entirely on the side of the diastereomer (*M*,7*S*)-**14**, bearing the C-7 acetamido substituent in an equatorial position, as indicated by the chemical shift of the relevant CH_3-protons at $\delta = 2.00$. On the other hand, the chemical shift for the protons of the axially positioned acetamido group in (*P*,7*S*)-**14** is distinctly high-field-shifted to $\delta = 1.54$ because it is influenced by the anisotropic effect of the aromatic A-ring.[5,11]

Scheme 4



Conclusions

In conclusion, we wish to note the following significant points arising from the study outlined herein: (1) We have demonstrated for the first time that the helimeric mixture of unnatural (−)-isocolchicine (**6**), characterized by two chiral elements, such as (−)-colchicine, readily cycloadds the superdienophilic singlet oxygen with high regioselectivity at the 7a,11-positions of the alkaloid. This methodology has already been used by us as one of the key steps in the synthesis of the allocolchicinoids *N*-acetylcolchinol *O*-methyl ether and androbiphenyline.[2] The approach of the dienophile occurred preferentially—in contrast to the reaction mode of (−)-colchicine—toward the anti π-face of the 7a,12-diene moiety of **6**, leading to the endoperoxides (*M*,7*S*)-**7** and (*M*,7*S*)-**8** with a 1:7 ratio in a total yield of 62%. (2) The triethylamine-induced transformation of the predominant endoperoxide **8** furnished a mixture of two probably interconverting epoxides (*M*,7*S*)-**10a** and (*M*,7*S*)-**10b**. (3) These on treatment with silica gel display some unique stereochemical aspects including an intramolecular transfer of axial into central chirality. Controlled by the chiral twist of the biaryl axis of the intermediate product (*M*)-**11**, formed by a retro-Mannich fragmentation, the configurationally stable (under the conditions employed) (*M*)-**11** reacts enantioselectively to yield an atropoenantiomerically pure colchicinoid (*M*,7*S*)-**12** characterized by an eight-membered oxocine B-ring.

Experimental Section

For general procedures, see ref 12. Standard vacuum techniques were used in handling of air-sensitive materials. Melting points are uncorrected. Solvents were dried and freshly distilled before use according to literature procedures. IR spectra: liquids were run as films, solids as KBr pellets. ^{1}H NMR spectra were recorded at 400 or 500 MHz, ^{13}C NMR spectra at 100 and 125 MHz; δ/ppm = 0 for tetramethylsilane, 7.24 for chloroform. Column chromatography: Purifications were carried out on silica gel 40 (40−60 mesh), flash chromatography; reactions were monitored by thin-layer chromatography (TLC) by using plates of silica gel (0.063−0.200 mm) or silica gel-60F_{254} microcards. Optical rotations were measured in $CHCl_3$ at 20 °C. UV spectra were recorded in MeOH.

Reaction of Isocolchicine (6) with Singlet Oxygen. A solution of the alkaloid **6**[4] (600 mg, 1.5 mmol) and the photosensitizer hematoporphyrin (20 mg) in $CHCl_3$ (250 mL) was saturated with oxygen and irradiated for 30 min in a Pyrex cell at 0 °C with an Osram Vialox NAV-TS 250 W high-pressure sodium lamp. The solvent was evaporated in vacuo below 30 °C, and the residue was purified by column chromatography (silica gel, column 12 × 4 cm, CH_2Cl_2/MeOH 9.8:0.2). Fractions 1 and 2 contained the cycloadducts **7** and **8** in an overall yield of 62%, which were recrystallized from CH_2Cl_2/*n*-hexane. **8** is a mixture of two conformers: *M*/*P*-ratio: 2:3. Fraction 3 contained **6** recovered in 18% yield.

Fraction 1: 7aα,11α-Epidioxy-7a,11-dihydro-isocolchicine (7): Yield 52 mg (8%), colorless crystals, mp 107−109 °C; IR (cm^{-1}, KBr): 3276 (NH), 1704 (C=O), 1661 (C=O); UV (MeOH): λ_{max} (lg ϵ): 262.8 nm (3.86), 214 (4.51); $[\alpha]^{20}_D = -270$ (c = 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.81 (m, 1H), 1.92 (m, 1H), 1.99 (s, 3H), 2.52 (dd, ^2J = 13.75 Hz, ^3J = 7.1 Hz, 1H), 2.85 (m, 1H), 3.53 (s, 3H), 3.62 (s, 3H), 3.82 (s, 3H), 3.84 (s, 3H), 4.38 (m, 1H), 5.22 (d, ^3J = 7.55 Hz, 1H), 5.83 (d, hidden, 1H), 5.85 (s, 1H), 6.16 (d, ^3J = 7.75 Hz, 1H), 6.48 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 23.30, 28.23, 30.24, 50.69, 55.21, 56.04, 60.87, 61.90, 84.24, 84.53, 107.55, 115.20, 118.62, 120.77, 132.84, 141.18, 148.57, 150.32, 150.88, 153.88, 169.81, 189.57. MS, *m*/*z* (%): 431 (52) [M$^+$], 413 (44), 387 (32), 372 (100). Anal. Calcd for $C_{22}H_{25}NO_8$: C, 61.25; H, 5.84. Found: C, 60.85; H, 5.86.

Fraction 2: 7aβ,11β-Epidioxy-7a,11-dihydro-isocolchicine (8): Yield 354 mg (54%), colorless crystals, mp 146−147 °C; IR (cm^{-1}, KBr): 3259 (NH), 1702 (C=O); UV (MeOH) λ_{max} (lg ϵ): 355.4 nm (3.48), 257.4 (4.19), 215 (4.50); $[\alpha]^{20}_D = -22$ (c = 0.32, $CHCl_3$); ^{1}H NMR ($CDCl_3$), (*P*)-conformer: δ 1.64 (s, 3H), 1.69 (m, 1H), 2.40 (m, 1H), 2.50 (m, 1H), 3.04 (m, 1H),

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3.55 (s, 3H), 3.62 (s, 3H), 3.85 (s, 3H), 3.90 (s, 3H), 4.68 (m, 1H), 4.72 (d, ^3J = 11 Hz, 1H), 5.25 (d, ^3J = 7.75 Hz, 1H), 6.06 (s, 1H), 6.28 (d, ^3J = 8 Hz, 1H), 6.62 (s, 1H); (*M*)-conformer: δ 1.85 (m, 1H), 1.94 (s, 3H), 2.51 (m, 1H), 2.65 (m, 2H), 3.70 (s, 3H), 3.74 (s, 3H), 3.84 (s, 6H), 4.12 (m, 1H), 5.32 (d, ^3J = 8.7 Hz, 1H), 5.90 (d, ^3J = 6.15 Hz, 1H), 5.95 (s, 1H), 6.43 (s, 1H), 6.72 (d, ^3J = 8.7 Hz, 1H); ^{13}C NMR ($CDCl_3$), (*P*)-conformer: δ 23.35, 29.33, 29.96, 49.98, 55.29, 56.13, 61.14, 61.64, 84.46, 84.46, 107.55, 115.60, 120.64, 122.90, 134.46, 141.49, 148.02, 151.03, 151.65, 153.92, 169.20, 189.90; (*M*)-conformer: δ 23.44, 30.64, 31.86, 50.53, 55.39, 55.92, 61.14, 61.27, 83.21, 84.82, 107.84, 109.35, 119.70, 119.80, 134.47, 141.50, 147.26, 152.27, 153.59, 154.14, 170.14, 190.74; MS, *m*/*z* (%): 431 (56) [M$^+$], 413 (38), 387 (100), 372 (81). Anal. Calcd for $C_{22}H_{25}NO_8$: C, 61.25; H, 5.84. Found: C, 60.87; H, 5.82.

Crystal Structure Determination of 7. A yellow, single crystal (ca. 0.12 × 0.52 × 0.76 mm) was investigated on a four-circle diffractometer (Enraf-Nonius CAD 4) by using graphite-monochromated Mo-K_α radiation at −50 °C, ω-scan mode; empirical formula: $C_{23}H_{27}Cl_2NO_8$, molecular mass 514.34, absorption coefficient μ = 0.29 mm^{-1}. The lattice constants of the orthorhombic unit cell, space group $P\,2_12_12_1$ (Z = 4, d_{calcd} = 1.302 mg mm^{-3}) were refined with the diffraction angles of 25 reflections in the 2θ range of 30−36° to furnish a = 952.0(1), b = 1010.7(1), c = 2726.0(5) pm. For the refinement, 4590 independent reflections out of 5265 measured reflections (R_{int} = 0.019) were used in the 2θ range of 4−50° ($0 \le h \le 11$, $0 \le k \le 12$, $0 \le l \le 32$, Friedel opposites measured). An empirical absorption correction was carried out,[16a] min and max. transmission: 0.61 and 1.00. The structure was solved by direct methods[16b] and subjected to full matrix least-squares refinement based on F^2 values,[16c] with anisotropic displacement factors for all heavier atoms (data/parameter = 13:1). The hydrogen atoms were included at idealized positions with fixed temperature factors using a riding model. There is one multiple disordered CH_2Cl_2 molecule in the unit cell. The absolute structure could be determined with the help of Flack's absolute structure parameter (−0.08(2)).[16d] Final reliability factors: R_1 = 0.050 for 3531 reflections with $I > 2\sigma(I)$, wR_2 = 0.123 ($w = 1/[\sigma^2(F_o)^2 + (0.066P)^2 + 0.1563P]$, with $P = ([F_o^2 + 2F_c^2]/3)$ for all data.[16e]

Cleavage of the Endoperoxide (*M*,7*S*)-8 with Triethylamine. To a solution of the endoperoxide **8** (215 mg, 0.50 mmol) in CH_2Cl_2 (10 mL) cooled to 0 °C was added a solution of triethylamine (70 μL, 0.5 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was kept at 0 °C for additional 30 min. Then the solvent was evaporated in vacuo and the crystalline residue suspended in a minimal amount of CH_2Cl_2 (approximately 1 mL). The suspension was filtered to give pale yellow crystals (191 mg, 89%). As indicated by ^{1}H NMR spectroscopy the product is a mixture of two isomers (*M*,7*S*)-**10a**/(*M*,7*S*)-**10b**, which probably interconverted in $CDCl_3$ solution. In DMSO-d_6 as the solvent the well focused signals for both isomers could be detected (ratio **10a**/**10b** 2:1).

[7S-(7α,7a*β*,11*β*)]-*N*-(6,7,10,11-Tetrahydro-11-hydroxy-1,2,3,9-tetramethoxy-10-oxo-5*H*-7a,11-epoxybenzo[*a*]heptalen-7-yl)acetamide (*M*,7*S*)-10a and [7S-(7α,7a*β*,10*β*)]-*N*-(6,7,10,11-Tetrahydro-10-hydroxy-1,2,3,9-tetramethoxy-11-oxo-5*H*-7a,10-epoxybenzo[*a*]heptalen-7-yl)acetamide (*M*,7*S*)-10b: Yield 191 mg (89%), pale yellow crystals, mp 132−133 °C; IR (cm^{-1}, KBr): 3600−3100 (OH, NH), 1710 (C=O), 1649 (C=O); UV (MeOH): λ_{max} (lg ϵ) = 349.2 nm (3.88), 249.4 (4.05), 209.8 (4.46); $[\alpha]^{20}_D$ = −74 (c = 0.29, $CHCl_3$).

10a: ^{1}H NMR (DMSO-d_6): δ 1.63 (m, 1H); 1.84 (s, 3H); 1.94 (m, 1H); 2.60 (m, 1H); 2.87 (m, 1H); 3.41 (s, 3H); 3.47 (s, 3H); 3.70 (s, 3H); 3.80 (s, 3H); 4.33 (m, 1H); 6.24 (s, 1H); 6.39 (s, 1H); 6.72 (s, 1H); 7.74 (s, 1H); 7.85 (d, ^3J = 8.5 Hz, 1H); ^{13}C NMR (DMSO-d_6): δ = 22.88, 29.89, 33.18, 54.82, 55.41, 55.72, 60.21, 60.29, 87.49, 106.33, 109.21, 116.76, 117.90, 129.63, 137.82, 140.62, 147.20, 149.55, 152.43, 153.02, 168.34, 188.86.

10b: ^{1}H NMR (DMSO-d_6) δ 1.78 (s, 3H); 1.81 (m, 1H); 1.94 (m, 1H); 2.60 (m, 2H); 3.61 (s, 3H); 3.70 (s, 3H); 3.74 (s, 3H); 3.84 (s, 3H); 3.99 (m, 1H); 5.83 (s, 2H); 6.79 (s, 1H); 7.50 (m, 1H); 7.74 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 22.78, 30.24, 33.04, 55.85, 58.24, 60.51, 60.97, 85.27, 103.61, 103.86, 108.80, 120.21, 122.16, 135.42, 140.89, 153.02, 154.47, 162.13, 163.19, 168.63, 192.74 (C-7 could not be detected, obviously hidden).

MS, *m*/*z* (%): 431 (100) [M$^+$]; 389 (46); 387 (34); exact mass calcd for $C_{22}H_{25}NO_8$ 431.1580, found 431.1578.

(*S*)-*N*-(3,7,8,9-Tetrahydro-2-hydroxy-4,11,12,13-tetramethoxy-3-oxocyclohepta[*a*][3]-benzoxocin-7-yl)acetamide (*M*,7*S*)-12. The hemiketals **10a/10b** (175 mg, 0.4 mmol) and silica gel (4.0 g, 40−60 mesh) were suspended in ethyl acetate (10 mL) and stirred at room temperature for 20 h in the absence of light. The suspension was filtered and the filter cake washed with ethyl acetate (6 × 150 mL). The fractions were combined, and the solvent was evaporated in vacuo. An amount of 133 mg (76%) of crude product was obtained, which was crystallized from CH_2Cl_2/diethyl ether (1:1). Yield 84 mg (48%), pale yellow crystals, mp 132−133 °C; IR (cm^{-1}, KBr): 3272 (NH), 1678 (C=O); UV (MeOH): λ_{max} (lg ϵ) = 389 nm (4.20), 334.8 (4.07), 252.4 (4.58), 206.2 (4.59); $[\alpha]^{20}_D$ = −112 (c = 0.28, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.80 (m, 1H); 2.01 (s, 3H); 2.14 (m, 1H); 2.37 (m, 1H); 2.70 (m, 1H); 3.58 (s, 3H); 3.85 (s, 3H); 3.86 (s, 3H); 4.10 (s, 3H); 5.56 (m, 1H); 6.43 (d, ^3J = 8.95 Hz, 1H); 6.55 (s, 1H); 7.42 (s, 1H); 8.17 (s, 1H); 8.72 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ 23.43, 30.08, 34.33, 56.16, 57.10, 61.00, 61.17, 84.98, 108.09, 116.33, 121.46, 125.30, 129.78, 136.14, 141.06, 150.86, 153.41, 153.53, 156.61, 159.71, 170.55, 170.83; MS, *m*/*z* (%): 431 (100) [M$^+$]; 389 (40); exact mass calcd for $C_{22}H_{25}NO_8$: 431.1580, found 431.1591.

Crystal Structure Determination of (*M*,7*S*)-12. A yellow single-crystal platelet (ca. 0.28 × 0.12 × 0.05 mm) was investigated on an IPDS area detector system (Stoe) using graphite-monochromated Mo-K_α radiation at −80 °C. Empirical formula: $C_{22}H_{25}NO_8 \cdot 0.625CH_2Cl_2$, molecular mass 484.51, absorption coefficient μ = 0.24 mm^{-1}. The lattice constants of the monoclinic unit cell, space group $P2_1$ (Z = 6, d_{calcd} = 1.346 g cm^{-3}) were refined with the diffraction angles of 8000 reflections in the θ range of 11−26° to furnish a = 1222.23(8), b = 1621.5(1), c = 1831.1(2) pm, β = 98.705(8)°. For the refinement, 16393 independent out of 43786 measured reflections (R_{int} = 0.066) were used in the 2θ range of 4.5−56.2° ($-16 \le h \le 15$, $-21 \le k \le 21$, $0 \le l \le 24$). A numerical absorption correction was carried out leading to transmission factors between 0.90 and 0.97. The structure was solved by direct methods[16b] and refined by full matrix least-squares methods based on F^2 values,[16c] with anisotropic displacement factors for all heavier atoms (data/parameter ratio = 19). The hydrogen atoms were included on idealized positions using a riding model with displacement factors taken as 1.2 (1.5 for CH_3) times the equivalent U of the corresponding C atoms. The phenyl rings were constrained to idealized geometry. There are three independent but very similar molecules in the asymmetric unit and 0.625 molecules dichloromethane per molecule on two crystallographic positions each occupied by 94.1(2)%. At one of them disorder is observed. The final residuals converged to: R = 0.0475 for 7888 observed reflections with $I > 2\sigma(I)$, wR_2 = 0.0884 for all data ($w = 1/[\sigma^2(F_o)^2 + (0.2P)^2]$, with $P = ([F_o^2 + 2F_c^2]/3)$.

The correctness of the absolute structure could be confirmed despite the use of Mo−Kα radiation due to the presence of the solvent chlorine atoms as anomalous scatterers as shown by refinement of the Flack parameter[16d] x = −0.06(6). For additional proof, the crystal was measured on a 4-circle diffractometer (CAD4, Nonius) with Cu−Kα radiation. Using these data the Flack parameter refined to 0.00(3), confirming the correctness of the absolute configuration. For the 10 most significant Bijvoet differences ($|F_c^2(hkl) - F_c^2(-h-k-l)| \ge 4\sigma(F^2)$) all observed signs agree with that of the calculated

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ones. As the accuracy of the atomic positions from refinement with the area detector data (Mo−Kα) is much better than that based on the 4-circle diffractometer data (Cu−Kα), the former results are further used only.

(±)-*N*-(3,7,8,9-Tetrahydro-2-hydroxy-4,11,12,13-tetramethoxy-3-oxocyclo-hepta[a][3]-benzoxocin-7-yl)acetamide (*rac*-12). The hemiketals **10a/10b** (20 mg, 0.046 mmol) were refluxed in dry xylene (5 mL) under argon for 2 h. The reaction leads nearly quantitatively to the main product *rac*-**12**, which is crystallized (5 mg) by slowly evaporating of the solvent. The data obtained by ^{1}H NMR spectroscopy are in agreement with those observed for compound (−)-**12**. $[\alpha]^{20}_D = 0$ ($c = 0.25$, $CHCl_3$).

(*S*)-*N*-(3,7,8,9-Tetrahydro-2,4,11,12,13-pentamethoxy-3-oxocyclohepta[*a*][3]benzoxocin-7-yl)acetamide (*M*,7*S*)-(14). A solution of the colchicinoid (*M*,7*S*)-**12** (50 mg, 0.12 mmol) in a mixture of CH_2Cl_2/CH_3OH 4:1 (5 mL) was treated twice with an excess of ethereal CH_2N_2, and after stirring the mixture at room temperature for 3 h, the solvent was evaporated in vacuo and the residue purified by column chromatography (silica gel, column 7.5 × 2 cm, CH_2Cl_2/MeOH 9.5:0.5). The product is a mixture of two conformers: *M*/*P*-ratio: 9:1. Yield 28 mg (54%), colorless amorphous powder, mp 118−119 °C. IR (cm^{-1}, KBr): 3274 (NH), 1687 (C═O). UV (MeOH): λ_{max} (lg ϵ) = 380.4 nm (4.18), 333.8 (4.10), 250.8 (4.56), 206.4 (4.58). $[\alpha]^{20}_D = -53$ ($c = 0.62$, $CHCl_3$). ^{1}H NMR ($CDCl_3$), (*M*) conformer: δ 1.82 (m, 1H); 2.00 (s, 3H); 2.15 (m, 1H); 2.39 (m, 1H); 2.70 (m, 1H); 3.53 (s, 3H); 3.73 (s, 3H); 3.82 (s, 3H); 3.86 (s, 3H); 4.03 (s, 3H); 5.58 (m, 1H); 6.56 (s, 1H); 6.92 (s, 1H); 7.05 (m, 1H); 7.98 (s, 1H); ^{13}C NMR ($CDCl_3$), (*M*) conformer: δ 23.21, 30.09, 33.92, 56.05 (2C), 56.89, 60.91, 61.09, 84.12, 108.30, 113.70, 119.05, 125.44, 125.59, 136.38, 140.95, 150.70, 152.87, 153.14, 157.42, 161.42, 170.87, 172.78. MS, *m*/*z* (%): 445 (28) [M^+]; 386 (100); exact mass calcd for $C_{23}H_{27}NO_8$: 445.1737, found 445.1737.

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Supporting Information Available: X-ray data and details for the X-ray data acquisition for (*M*,7*S*)-**7** and (*M*,7*S*)-**12**, ^{1}H NMR and ^{13}C NMR spectra of compounds **10a10b**, (*M*,7*S*)-**12**, rac-**12**, and (*M*,7*S*)-**14**. ^{1}H NMR spectra of (*M*,7*S*)-**12** and rac-**12** in the presence of 3 equiv of quinine as chiral solvating agent. This material is available free of charge via the Internet at http://pubs.acs.org.

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