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Saludimerines A and B, Novel-Type Dimeric Alkaloids with Stereogenic Centers and Configurationally Semistable Biaryl Axes

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The first biarylic bis-morphinanedienone alkaloids, saludimerines A (**3a**) and B (**3b**), isolated from a tree of *Croton flavens* (Euphorbiaceae) are described. These naturally occurring dimers of the known alkaloid salutaridine are joined together via a rotationally hindered biaryl axis, giving rise to atropo-diastereomers that are configurationally stable at room temperature but slowly interconvert in methanolic solution within several days. Their structures were established by spectroscopic methods and by partial synthesis, which was achieved by a highly atropo-diastereoselective biomimetic oxidative coupling of the monomeric precursor, salutaridine. Their axial configurations were elucidated by circular dichroism (CD) investigations, which succeeded despite the fact that the two atropo-diastereomers exhibit near-identical CD spectra. This remarkable phenomenon was rationalized by quantum chemical CD calculations. The configurational assignment of saludimerines A (**3a**) as *P*-axial and B (**3b**) as *M* was corroborated by atropisomer-specific NOE interactions between protons of the one molecular half with nuclei in the other.

Introduction

The genus *Croton* (Euphorbiaceae) is abundant in the tropical regions of America and Africa. Phytochemical investigations of various species led to the identification of terpenoids (mainly phorbol esters[1]) and numerous benzylisoquinoline-derived alkaloids.[2] *Croton flavens* from Jamaica contains morphinanedienone alkaloids (flavinine, sinoacutine, norsinoacutine, flavinantine).[3] From a Columbian plant of this species, the proaporphine amuronine was isolated.[4] Our previous investigations on the leaves from *C. flavens* originating from Barbados led to the isolation of the tetrahydroprotoberberine alkaloids scoulerine and coreximine as well as the morphinanedienones salutaridine, salutarine, sebiferine, norsinoacutine, and flavinantine.[5] Some time ago, we reported on the new phenanthrene crotoflavol from *C. flavens*.[6]

Croton species have attracted considerable interest, since morphinanedienone alkaloids do not necessarily occur enantiomerically pure in these plants. Thus, Amaral and Barnes[7] reported the isolation of salutarine, a mixture of the enantiomeric alkaloids salutaridine (**1**) and sinoacutine (*ent*-**1**), from *C. salutaris*. In our recent investigations on three trees of *C. flavens* we found that one tree produced enantiopure salutaridine (**1**), accompanied by norsinoacutine (*ent*-**2**), the enantiomer of norsalutaridine (**2**, an alkaloid from *C. salutaris*[8]), whereas two other trees contained the enantiomeric mixture salutarine.[8]

In continuation of our research on alkaloids from *C. flavens* we have now analyzed another tree from our greenhouse, originally indigenous to Barbados. In this paper, we describe the isolation and stereochemical characterization of the novel-type alkaloids saludimerine A (**3a**) and saludimerine B (**3b**), the first axially chiral biarylic morphinanedienone "dimers",[9] from the leaves of that tree, along with the known "monomeric" alkaloids salutaridine (**1**) and norsinoacutine (*ent*-**2**) (Figure 1).

Results and Discussion

Isolation of the Alkaloids. The alkaloid fraction separated from the defatted MeOH extract of the leaves

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(1) Weber, J.; Hecker, E. *Experientia* **1978**, *34*, 679−682.
(2) Farnsworth, N. R.; Blomster, R. N.; Messmer, W. M.; King, J. C.; Persinos, G. J.; Wilkes, D. J. *Lloydia* **1969**, *32*, 1−28.
(3) Stuart, K.; Chambers, C.; Byfield, D. *J. Chem. Soc. C* **1969**, 1681−1684.
(4) Charris, J.; Dominguez, J.; de la Rosa, C.; Caro, C. *Biochem. Syst. Ecol.* **2000**, *28*, 795−797.
(5) Eisenreich, W. J.; Höfner, G.; Bracher, F. *Nat. Prod. Res.* **2003**, *17*, 437−440.
(6) Eisenreich, W. J.; Bracher F. *Nat. Prod. Lett.* **2001**, *15*, 147−150.
(7) Amaral, A.; Barnes, R. *Phytochemistry* **1998**, *47*, 1445−1447.
(8) Barnes, R. A.; Soeiro, O. M. *Phytochemistry* **1981**, *20*, 543−544.
(9) Strictly speaking, these compounds should rather be addressed as "dehydrodimers"; still, for reasons of simplicity, the term "dimer" (like also "monomer" for the precursor) will be used throughout this paper.

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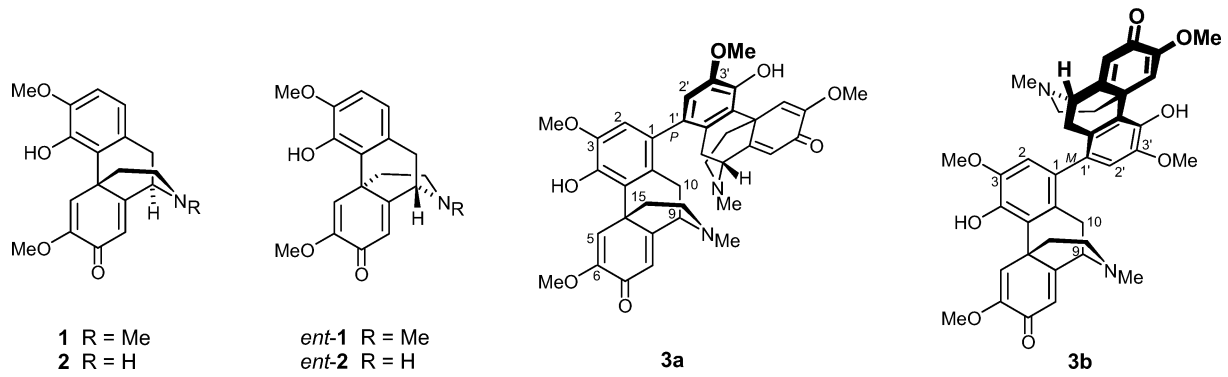

FIGURE 1. Secondary metabolites from *C. flavens*: the monomeric alkaloids salutaridine (**1**) and norsinoacutine (*ent*-**2**) and the axially chiral "dimers", saludimerines A (**3a**) and B (**3b**).

TABLE 1. ^{1}H NMR and ^{13}C NMR Spectral Data of Salutaridine (1), Saludimerine A (3a), and Saludimerine B (3b), Recorded in $CDCl_3$

	salutaridine (**1**)		saludimerine A (**3a**)		saludimerine B (**3b**)	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	6.65, d, 1H (8.3)	118.7		131.6		131.1
2	6.74, d, 1H (8.3)	109.6	6.59, s, 1H	110.1	6.44, s, 1H	111.1
3		145.5		145.6		145.6
4		143.5		142.8		142.8
5	7.60, s, 1H	120.7	7.56, s, 1H	120.3	7.53, s, 1H	120.5
6		150.9		151.1		151.1
7		181.4		181.5		181.5
8	6.32, s, 1H	122.1	6.30, s, 1H	122.1	6.24, s, 1H	122.4
9	3.69, d, 1H (5.3)	61.1	3.57, d, 1H (5.1)	60.6	3.51, d, 1H (2.9)	61.0
10α	2.97, dd, 1H (17.6, 5.6)	32.6	2.26, m, 1H	31.1	2.66, m, 2H	33.0
10*β*	3.34, d, 1H (17.6)		2.84, d, 1H (18.3)			
11		129.6		127.8		127.1
12		124.0		124.6		124.7
13		43.7		43.9		44.0
14		161.8		161.5		161.3
15	1.77, m, 1H	37.7	1.78, m, 1H	37.6	1.77, m, 1H	37.5
	2.39, m, 1H		2.46, m, 1H		2.37, d, 1H (12.6)	
16	2.49, dd, 1H (12.5, 3.1)	47.0	2.46, m, 1H	47.3	2.57, m, 2H	47.1
	2.61, dd, 1H (12.5, 3.0)		2.62, dd, 1H (12.1, 4.0)			
N-CH_3	2.44, s, 3H	41.6	2.29, s, 3H	41.7	2.33, s, 3H	42.0
3-OCH_3	3.86, s, 3H	56.3	3.91, s, 3H	56.4	3.77, s, 3H	56.4
6-OCH_3	3.74, s, 3H	54.8	3.78, s, 3H	54.9	3.70, s, 3H	55.0

of *C. flavens* by extraction with 5% acetic acid was subjected to column chromatography on silica gel using a CH_2Cl_2−MeOH gradient system.

Some early fractions gave large amounts of the known[10] enantiopure alkaloid salutaridine (**1**), which was identified by comparison (α_D and chromatography on a chiral phase) with an authentic sample. One of the less polar fractions gave, after repeated chromatography, the likewise known[10] alkaloid norsinoacutine (*ent*-**2**), as well as the first dimeric morphinanedienone saludimerine A (**3a**). The other dimer, saludimerine B (**3b**), was obtained from the following fractions.

Structural Elucidation. The two new alkaloids, **3a**, $[\alpha]^{20}_D$ −219 (*c* 1.7, MeOH), and **3b**, $[\alpha]^{20}_D$ −154 (*c* 1.4, MeOH), showed very similar spectroscopic data. Both compounds gave HR-MS values that suggested a molecular formula of $C_{38}H_{40}N_2O_8$. Surprisingly, the NMR spectra of each compound exhibited only 20 hydrogen and 19 carbon resonances. These results clearly suggested that the two new alkaloids should be constitutionally and configurationally symmetric dimeric compounds. Since the NMR data of both **3a** and **3b** revealed very strong similarities with those of the main alkaloid salutaridine (**1**, Table 1), the new alkaloids were assumed to be symmetric dimeric morphinanedienone alkaloids.

In the ^{1}H NMR spectra of **3a** and **3b**, the resonances of the aliphatic protons were quite similar to those recorded for the monomer salutaridine (**1**). The chemical shifts of the singlets at the cyclohexadiene moiety could also be clearly correlated by HMBC and NOE experiments. In the aromatic parts of **3a** and **3b**, however, we found only three singlets, compared to two singlets and two doublets in **1**. In each of the ^{13}C NMR spectra of the dimers, one tertiary aromatic carbon was replaced by a quaternary aromatic carbon. These findings can only be explained by the presence of dimeric morphinanedienones with a biaryl axis joining the two monomeric portions. From the presence of three singlets for the aromatic protons of both dimers, we could conclude that the two morphinanedienone subunits had to be connected either from C-1 to C-1′ or from C-2 to C-2′. NOE experiments showed that in both dimers the 3-methoxy group is neighbored by an aromatic hydrogen at C-2. Conse-

(10) Chambers, C.; Haynes, L. J.; Stuart, K. L. *J. Chem. Soc., Chem. Commun.* **1966**, 449.

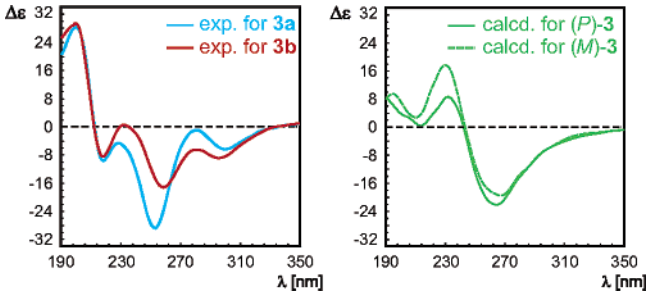

FIGURE 2. Experimental CD spectra of the two atropo-diastereomeric compounds **3a** and **3b** (left) and CD spectra calculated for (*P*)-**3** and (*M*)-**3** (right).

quently, both new alkaloids had to be 1,1′-connected. These interpretations were fully confirmed by extensive HMBC experiments.

Partial Syntheses and Equilibration Experiments. The spectroscopic data discussed above clearly suggested that both **3a** and **3b** are symmetrical dimers of morphinanedienones. From the fact that salutaridine (**1**) itself was also found in the same extract, it was obvious that this might be the monomeric congener of one or both of the new dimers. Since the proposed dimeric structures are formal products of an oxidative phenolic coupling, we examined the oxidation of enantiomerically pure salutaridine under different conditions. Mild oxidation of salutaridine with $AgNO_3$ at 0 °C, as described for the synthesis of a 1,1′-dimer of the morphinane alkaloid sinemonine,[11] gave a product that was identical to saludimerine A (**3a**) in all respects. An alternative oxidation method with potassium ferricyanide in alkaline solution, as described for the synthesis of pseudomorphine from morphine,[12] again stereoselectively gave the dimer **3a**, this time accompanied by minor amounts of **3b**.

Finally, we found that pure **3a** could be converted to a 1:1 mixture of **3a** and **3b** by heating the compound above the melting point or by storing it in methanolic solution for several days. Under the same conditions, **3b** also gave an equilibration to a mixture of **3a** and **3b**.

All these results led to the conclusion that the two new alkaloids, **3a** and **3b**, are constitutionally and configurationally symmetric 1,1′-coupled dimers of the alkaloid salutaridine. They have the same constitution and obviously are atropo-diastereomers with semistable stereogenic axes.

CD Measurements. As for other axially chiral biarylic natural products,[13] an investigation of the circular dichroism (CD) behavior of the two atropo-diastereomeric dimers, **3a** and **3b**, appeared to be the method of choice for an assignment of the respective axial configurations. Surprisingly, however, the CD spectra of **3a** and **3b** showed nearly identical curve shapes (Figure 2), which is in a sharp contrast to the CD behavior expected for atropo-diastereomers: Usually, the biaryl system with the two aromatic chromophores largely dominates the chiroptical behavior, so that the opposite stereochemical array at the biaryl axis of atropo-diastereomers leads to largely different, usually even near-opposite CD spectra,[14−16] depending on the chiroptical influence of other stereogenic parts of the system.

The close similarity of the CD spectra of **3a** and **3b** was apparently a consequence of the strong, dominating CD effects of the "centro-chiral" part of the molecule, viz. the stereochemically distorted dienone chromophore (whose absolute configuration was known anyhow from the above-described semisynthetic preparation from the respective monomeric halves). This spectral similarity was not likely to make it easy to deduce the absolute axial configuration using, e.g., empirical methods such as the exciton chirality approach.[17]

CD Calculations. Although at first sight the CD spectra of **3a** and **3b** did not provide substantial differences for a determination of the absolute axial configurations, quantum chemical CD calculations[18−20] on these two interesting compounds seemed rewarding, at least to explain and confirm this unexpected CD similarity. Such calculations had previously proved to be a particularly valuable tool for configurational assignments on other—atropo-enantio- or -diastereomeric—axially chiral compounds.[21−24]

Due to the flexibility and large molecular size of **3a** and **3b**, the CD calculations were based on MD simulations using the Tripos[25] force field. In both cases, the simulations were carried out for 500 ps, recording the structure every 0.5 ps for further calculations. For the 1000 structures thus collected, single CD spectra were calculated. The computed spectra were averaged arithmetically over the trajectory to give the theoretical overall spectra. To take into account a systematic shift of the calculated CD spectra, a "UV correction" was carried out as introduced earlier.[18] Interestingly, the calculations provided the same striking similarity of the CD curves obtained for the two atropo-diastereomers as the experimental CD measurements (Figure 2).

(11) Minawikawa, J.-I.; Iijima, I.; Brossi, A. *Heterocycles* **1978**, *10*, 79−84.
(12) Bentley, K. W.; Dyke, S. F. *J. Chem. Soc.* **1959**, 2574−2577.
(13) Bringmann, G.; Günther, C.; Ochse, M.; Schupp, O.; Tasler, S. *Prog. Chem. Org. Nat. Prod.* (Herz, W., Falk, H., Kirby, G. W., Moore, R. E., Tamm, C., Eds.) Wien **2001**, *82*, 1−249.
(14) Govindachari, T. R.; Nagarayan, K.; Parthasarathy, P. C.; Rajagopalan, T. G.; Desai, H. K.; Chen, S. L.; Nakanishi, K. J. *Chem. Soc., Perkin Trans. 1* **1974**, *12*, 1413−1417.
(15) Bringmann, G.; Teltschik, F.; Michel, M.; Busemann, S.; Rückert, M.; Haller, R.; Bär, S.; Robertson, A.; Kaminsky, R. *Phytochemistry* **1999**, *52*, 321−332.
(16) Bringmann, G.; Dreyer, M.; Faber, J.; Dalsgaard, P. W.; Staerk, D.; Jaroszewski, J.; Ndangalasi, H.; Mbago, F.; Brun, R.; Reichert, M.; Maksimenka, K.; Brøgger-Christensen, S. *J. Nat. Prod.* **2003**, *66*, 1159−1165.
(17) Nakanishi, K.; Berova, N.; Woody, R. W. In *The Exciton Chirality Method in Circular Dichroism, Principles and Applications*; Oxford University Press: Oxford, 1994.
(18) Bringmann, G.; Busemann, S. In *Natural Product Analysis*; Schreier, P., Herderich, M., Humpf, H. U., Schwab, W., Eds.; Vieweg: Wiesbaden, 1998; pp 195−212.
(19) Bringmann, G.; Mühlbacher, J.; Messer, K.; Dreyer, M.; Ebel, R.; Nugroho, B. W.; Wray, V.; Proksch, P. *J. Nat. Prod.* **2003**, *66*, 80−85.
(20) Meyring, M.; Mühlbacher, J.; Messer, K.; Kastner-Pustet, N.; Bringmann, G.; Mannschreck, A.; Blaschke, G. *Anal. Chem.* **2002**, *74*, 3726−3735.
(21) Bringmann, G.; Tasler, S.; Endress, H.; Kraus, J.; Messer, K.; Wohlfarth, M.; Lobin, W. *J. Am. Chem. Soc.* **2001**, *123*, 2703−2711.
(22) Bringmann, G.; Kraus, J.; Menche, D.; Messer, K. *Tetrahedron* **1999**, *55*, 7563−7572.
(23) Bringmann, G.; Pabst, T.; Henschel, P.; Kraus, J.; Peters, K.; Peters, E.-M.; Rycroft, D. S.; Connolly, J. *J. Am. Chem. Soc.* **2000**, *122*, 9127−9133.
(24) Bringmann, G.; Mühlbacher, J.; Reichert, M.; Dreyer, M.; Kolz, J.; Speicher, A. *J. Am. Chem. Soc.* **2004**, *126*, 9283−9290.
(25) SYBYL: Tripos Associates, 1699 Hanley Road, Suite 303, St. Louis, MO, 63144.

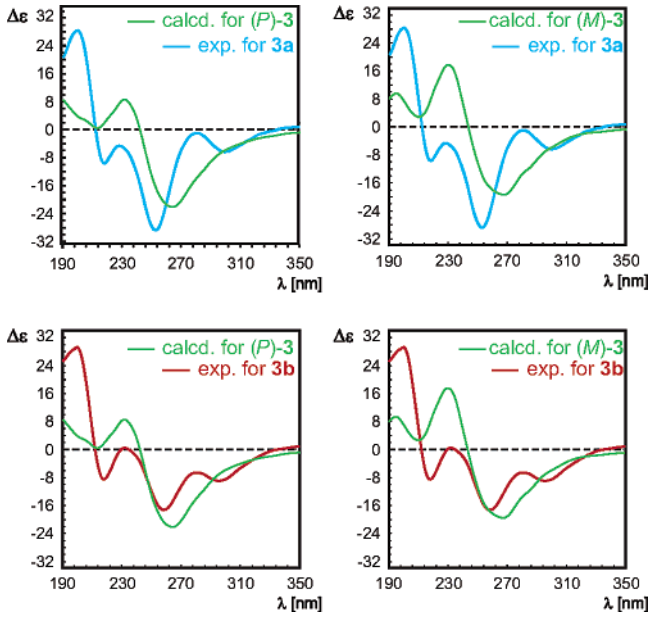

FIGURE 3. Comparison of the two calculated CD spectra of **3a** and **3b** with the experimental ones.

Besides the good reproduction of the similarity of the two atropo-diastereomers, the calculated CD spectra moreover showed a very good agreement with the spectra obtained experimentally (Figure 3). Still, as a consequence of this near-identical behavior, the CD curves of **3a** and **3b** did not provide a substantial difference to permit to clearly attribute a pairwise match of one experimental with one theoretical CD spectrum as necessary for the assignment of the absolute configuration, due to the high similarity of *all four* spectra shown in Figure 3.

Difference CD Spectra. If, however, it was indeed the prevailing "centro-chiral chromophore" that dominated the CD spectra of both **3a** and **3b**, and if this CD contribution was identical for both molecules (both have the same dienone chromophore with the same absolute configuration at the respective stereogenic centers), then it should be possible to eliminate this dominant centrochiral CD contribution arithmetically, by subtraction–additivity of the different contributions provided.

To test the assumption, we performed a thought experiment that had already been applied successfully in a similar way earlier, yet, for a case of dominant *axially* chiral CD contributions, viz. in the field of michellamines.[26] These dimeric naphthylisoquinoline alkaloids contain three biaryl axes (one of which is subject to rapid rotation while the other two ones are rotationally hindered and thus stereogenic) and four stereogenic centers. In the case of the saludimerines, one can imagine that it might be possible to eliminate the centrochiral part of the CD spectra (i.e., the one derived from the two monomeric salutaridine moieties) to give the axially chiral contribution (as arising from the combination of the two monomeric halves), by subtracting the CD spectrum of **3b** from that of **3a** or that of **3a** from **3b**; see Figure 4.

(26) Bringmann, G.; Gulden, K.-P.; Hallock, Y. F.; Manfredi, K. P.; Cardellina, J. H., II; Boyd, M. R.; Kramer, B.; Fleischhauer, J. *Tetrahedron* **1994**, *50*, 7807–7814.

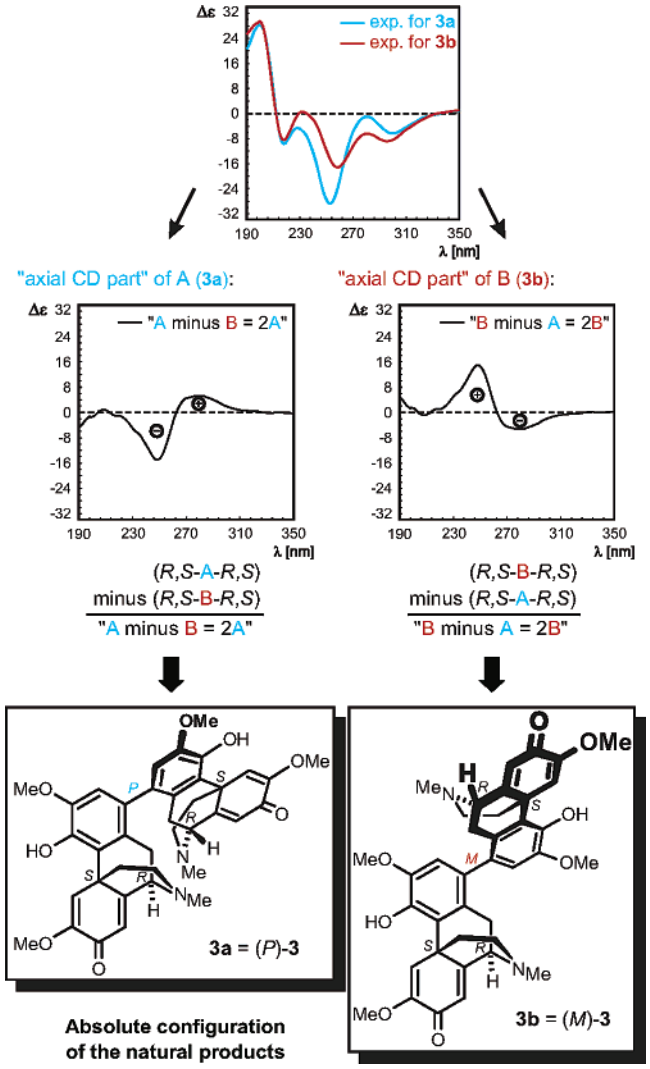

FIGURE 4. Numerically subtracted CD spectra composed of saludimerine A (**3a**) minus saludimerine B (**3b**) (left) and saludimerine B (**3b**) minus saludimerine A (**3a**).

Since the axial chromophore of **3a** (briefly: $axis_A$) is oppositely configured as compared to that of **3b** ($axis_B$), their CD contributions should likewise be opposite or, mathematically, "$axis_A = -(axis_B)$". This means that if one subtracts the CD spectrum of **3b** from the one of **3a**, the centrochiral part, which is identical in both molecular halves, should be calculatorily eliminated and what would remain is "$axis_A - axis_B = 2 \times axis_A$". In a similar way, if the spectrum of **3a** is subtracted from the one of **3b**, ideally "$2 \times (axis_B)$" should result or, in other words, exactly the contribution of the axis of **3b**, just enhanced by a factor of 2. The resulting difference CD spectra are displayed in Figure 4 (center, left and right) and can now, due to the C_2-symmetry of the molecules, be interpreted by means of the exciton chirality[17] method: The arithmetically "isolated" CD contribution of $axis_A$ provides a nearly ideal "positive couplet" (i.e., the first peak viewed from longer wavelengths is positive, and the second one negative, see Figure 4, left), and **3a** should thus be (*P*)-configured while, following an analogous argumentation and interpretation, the $axis_B$ of compound **3b** has to be (*M*)-configured (Figure 4, right).

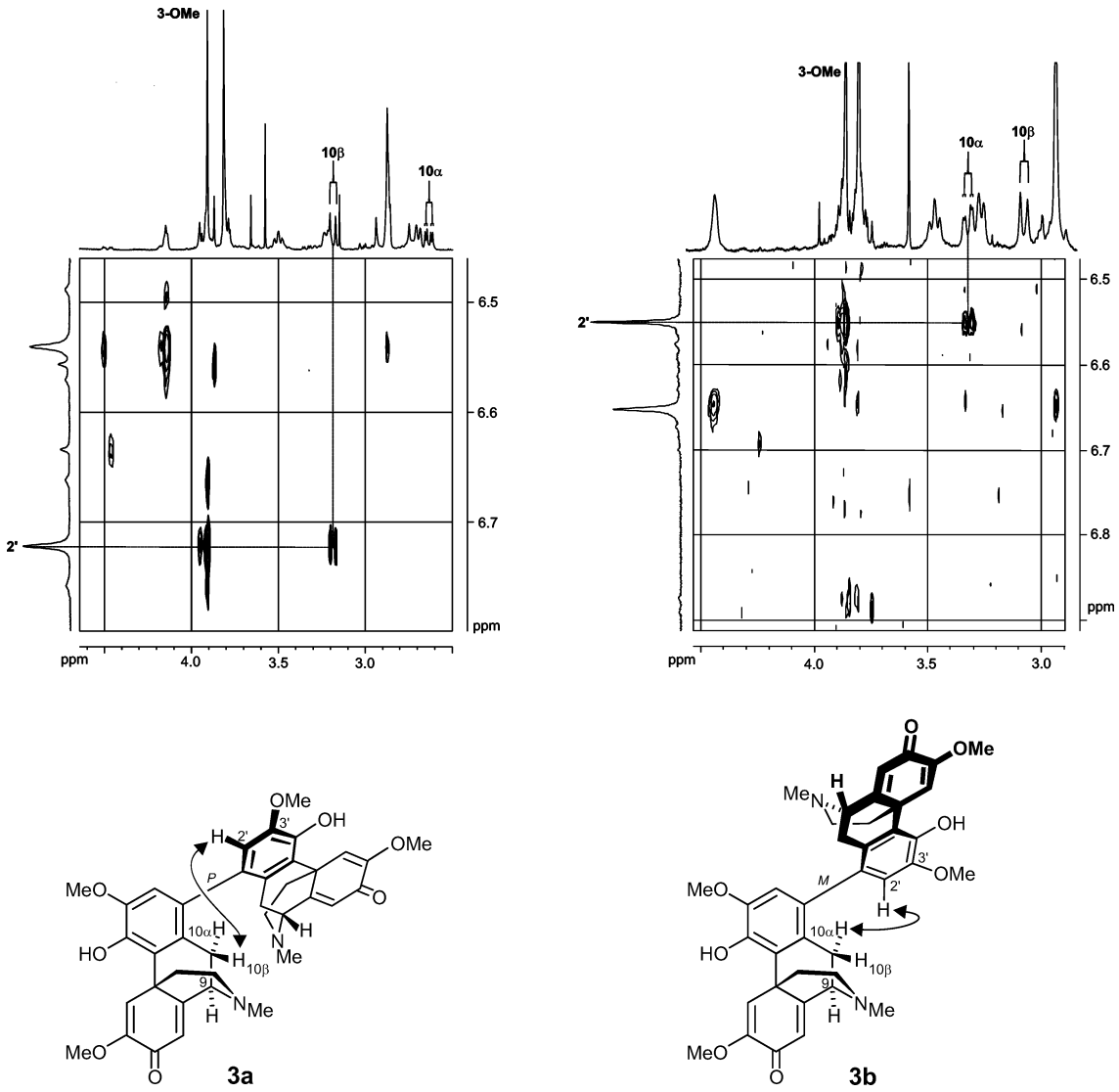

FIGURE 5. Selected ROESY interactions for the determination of the absolute configuration at the axis of saludimerines A and B.

NMR Experiments for the Determination of the Absolute Axial Configuration. This assignment of the absolute axial configurations of saludimerines A and B by this CD approach was further tested and finally confirmed by 2D-ROESY-NMR experiments (Figure 5), aiming at the identification of diagnostically valuable NOE interactions specific for the one or the other atropodiastereomer,[27−29] by taking advantage of the presence of diastereotopic protons at C-10. If specifically interacting with H-2′, these might give a clear assignment of the relative axial configuration and, in connection with the known absolute configuration at the stereogenic centers, also of the *absolute axial* configuration.

Initial ^{1}H NMR measurements in deuteriochloroform unfortunately resulted in only poorly resolved spectra with fully overlapping signals of H-10α and H-10*β*, due to their largely isochronous character. This situation did not permit us to distinguish between ROESY correlations of H-2′ with H-10α or with H-10*β*.

As in earlier cases,[30] the initially too small diastereotopic differentiation of the two protons at C-10 could fortunately be significantly increased by variation of the solvent. The best results were obtained by addition of deuterated trifluoroacetic acid (TFA) to the NMR sample, probably due to protonation (or, rather, to deuteration) of the basic nitrogen atom.

As the first consequence of this diastereotopic differentiation, the signals of the protons at C-10 were now unequivocally assigned as H-10α and H-10*β* by COSY, 1D-NOE, and homodecoupling ^{1}H NMR experiments. H-10*β* appeared as a doublet with a coupling constant typical of geminal protons (^2J = 19.55 Hz), H-10α displayed an additional ^3J-coupling of 6.7 Hz to H-9 because of a dihedral angle of 36°, while H-10*β* and H-9

(27) Bringmann, G.; Zagst, R.; Reuscher, H.; Aké Assi, L. *Phytochemistry* **1992**, *31*, 4011−4014.
(28) Hallock, Y. F.; Manfredi, K. P.; Blunt, J. W.; Cardellina, J. H., II; Schäffer, M.; Gulden, K.-P.; Bringmann, G.; Lee, A. Y.; Clardy, J.; François, G.; Boyd, M. R. *J. Org. Chem.* **1994**, *59*, 6349−6355.
(29) Bringmann, G.; Koppler, D.; Scheutzow, D.; Porzel, A. *Magn. Reson. Chem.* **1997**, *35*, 297−301.
(30) Bringmann, G.; Saeb, W.; Koppler, D.; François G. *Tetrahedron* **1996**, *52*, 13409−13418.

showed a dihedral angle of 80° according to AM1 calculations and thus gave no coupling in proton NMR. With the two diastereotopic protons at C-10 unequivocally assigned and sufficiently differentiated, detailed ROESY experiments now clearly revealed a correlation of the aromatic proton H-2′ of the one-half of this C_2-symmetric dimer "over the axis" with H-10β located at the other half of this dimer in the case of saludimerine A, thus unambiguously confirming the axis to be (*P*)-configured in this atropo-diastereomer, and with H-10α in saludimerine B, which was thus corroborated to be (*M*)-configured (Figure 5).

Conclusions

In conclusion, we describe here the first dimeric biaryl-type morphinanedienone alkaloids from plants. Only a dimer containing a biaryl ether linkage between the two morphinanedienone monomers, salutadimerine, had previously been described as a constituent of *Papaver pseudo-orientale* and *Papaver lasiothrix*.[31] The structures of saludimerine A (**3a**) and saludimerine B (**3b**) were assigned by extensive NMR experiments, by combined experimental and computational CD experiments, and by partial synthesis.

We can exclude that these dimers were formed as artifacts during extraction and fractionation, since other *Croton* species from our greenhouse, which also contain substantial amounts of the monomer salutaridine, have been worked up in the same manner, but not even traces of the dimers were found in their extracts.

Experimental Section

Plant Material and Extraction Procedures. Leaves were obtained from a tree of *C. flavens* L. originating from Barbados and cultivated under greenhouse conditions at the Department of Pharmacy, University of Munich.

Fresh leaves (885 g) were treated with liquid nitrogen and crushed. The fragments were suspended in MeOH and homogenized with an Ultra Turrax. The mixture was subsequently extracted in a Soxhlet apparatus with MeOH (1.5 L) for 24 h. Evaporation of the solvent gave a brown gum, which was taken up in 5% acetic acid (1 L) and defatted by extraction with *n*-hexane. The aqueous solution was adjusted to pH 8−9 with Na_2CO_3 and extracted with CH_2Cl_2 (4 × 750 mL). After concentration under reduced pressure, the residue (crude alkaloid fraction, 3.3 g) was fractionated by column chromatography on silica gel (500 g) using a gradient system CH_2Cl_2/MeOH (99:1, 98:2, 97:3, 95:5, 90:10, 50:50; 1 L each; fractions of 70 mL).

Fractions 31−42 gave salutaridine (**1**, 405 mg).

Fractions 67−70 were combined and the solvent evaporated. The residue (284 mg) was subjected to column chromatography on silica gel (100 g) with EtOAc/MeOH/ethyldimethylamine (85:10:5) to give the known alkaloid norsinoacutine (*ent*-**2**, 39 mg) and the new metabolite saludimerine A (**3a**, 90 mg).

Fractions 71−76 were combined and evaporated. The residue (230 mg) was subjected to column chromatography on silica gel (100 g), again with EtOAc/MeOH/ethyldimethylamine (85:10:5) as the eluent, this time followed by a second purification on silica gel (100 g) with CH_2Cl_2/MeOH (90:10) to give saludimerine B (**3b**, 41 mg).

Salutaridine (**1**) and norsinoacutine (*ent*-**2**) were identified by comparison with authentic samples isolated from other *Croton* plants.[5]

Saludimerine A (3a): white solid; mp 220 °C dec; $[\alpha]^{20}_D$ −219 (*c* 1.7, MeOH); IR (KBr) 3423, 2935, 2843, 1670, 1642, 1618, 1470, 1439 cm^{-1}; UV−vis (MeOH) λ (log ϵ) 241 (4.56), 283 (4.08); CD (*c* 0.04, EtOH) λ_{max} ($\Delta\epsilon$) 199 (24.4), 218 (−8.6), 228 (−4.0), 253 (−26.6), 280 (−1.4), 299 (−5.9); ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) see Table 1; HRMS calcd for $C_{38}H_{40}N_2O_8$ 652.2785, found 652.2759.

Saludimerine B (3b): white solid; mp 225 °C dec; $[\alpha]^{20}_D$ −154 (*c* 1.4, MeOH); IR (KBr) 3434, 2938, 2840, 1668, 1638, 1611, 1471 cm^{-1}; UV−vis (MeOH) λ (log ϵ) 244 (4.54), 283 (4.07); CD (*c* 0.025, EtOH) λ_{max} ($\Delta\epsilon$) 200 (44.5), 218 (−12.9), 232 (0.7), 258 (−26.2), 281 (−10.0), 295 (−13.6);^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) see Table 1; HRMS calcd for $C_{38}H_{40}N_2O_8$ 652.2785, found 652.2781.

Partial Syntheses. Method A. A solution of 34 mg (0.10 mmol) salutaridine (**1**) in 50 mL of 50% EtOH was cooled to 0 °C and treated with a solution of 27 mg of $AgNO_3$ in 1 mL of water. After the mixture was stirred for 30 min at 0 °C, 1 mL of saturated NaCl solution was added; the mixture was then adjusted to pH 8−9 by addition of Na_2CO_3 and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was evaporated. Column chromatography on silica gel (50 g) with CH_2Cl_2/MeOH (90:10) gave 20 mg (59%) of a white solid, which was identical to saludimerine A (**3a**) in all respects, accompanied by minor amounts of **3b**.

Method B. A 50 mg (0.15 mmol) sample of salutaridine (**1**) was dissolved in a solution of 20 mg of KOH in 10 mL of water with gentle heating. After the mixture was cooled to room temperature, a solution of 60 mg (0.18 mmol) potassium ferricyanide in 7 mL of water was added dropwise during 30 min. After another 30 min, the mixture was adjusted to pH 9 with 5% aqueous acetic acid and extracted with CH_2Cl_2 (3 × 50 mL). Chromatographic workup, as described above for fractions 71−76, gave 14 mg (28%) of saludimerine A (**3a**) and 9 mg (18%) of saludimerine B (**3b**).

NMR Experiments for the Assignment of the Axial Configurations. For measuring the ROESY interactions diagnostic of the relative configuration at the central biaryl axis, the samples of the two diastereomeric alkaloids were dissolved in 700 μL of deuteriochloroform, to each of which 10 μL of deuterated trifluoroacetic acid was added.

Computational Methods. The molecular dynamics simulations were performed using the Tripos force field as implemented in the molecular modeling package Sybyl 6.5,[25] using a time step of 0.5 fs. The molecule was weakly coupled to a thermal bath,[32] with a temperature relaxation time $\tau = 0.1$ s. The wave functions for the calculation of the rotational strengths for the electronic transitions from the ground state to excited states were obtained by CNDO/S-CI[33,34] calculations, in which the CI expansion takes into account the ground state and all n and π orbitals. These calculations were carried out using the BDZDO/MCDSPD[34] program package. For a better comparison of the theoretical CD spectrum with the experimental one, a Gaussian band shape function was generated over the calculated rotational strength values.

(31) Sariyar, G.; Freyer, A. J.; Guinaudeau, H.; Shamma, M. *J. Nat. Prod.* **1990**, *53*, 1383−1386.

(32) Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J. R. *J. Chem. Phys.* **1984**, *81*, 3684−3690.

(33) Downing, J. W. Program package BDZDO/MCDSPD, Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO; modified by Fleischhauer, J.; Schleker, W.; Kramer, B.; ported to Linux by Gulden, K.-P.

(34) Del Bene, J.; Jaffé, H. H. *J. Chem. Phys.* **1968**, *48*, 1807−1813.

(35) Gaussian 98 (Revision A.7). Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. Gaussian, Inc., Pittsburgh, PA, 1998.

Quantum chemical calculations were performed on i686-LinuX workstations using the Gaussian 98[35] program package. Input geometries were obtained using the TRIPOS force field as implemented within SYBYL 6.7.1[25] on a Silicon Graphics OCTANE (R10000).

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Supporting Information Available: General Experimental Section and ^{1}H and ^{13}C NMR spectra for **3a** and **3b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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