Chamazulene Carboxylic Acid and Matricin: A Natural Profen and Its Natural Prodrug, Identified through Similarity to Synthetic Drug Substances

Mai Ramadan,[†] Susanne Goeters,[†] Bernhard Watzer,[‡] Eva Krause,[§] Klaus Lohmann,[⊥] Rudolf Bauer,[∥] Bernd Hempel,[▽] and Peter Imming*[,§]

Institut fuer Pharmazeutische Chemie, Philipps-Universitaet, 35032 Marburg, Germany, Zentrum fuer Kinder- und Jugendmedizin, Philipps-Universitaet, 35037 Marburg, Germany, Institut fuer Pharmazeutische Biologie, Heinrich-Heine-Universitaet, 40225 Duesseldorf, Germany, Institut fuer Pharmazeutische Wissenschaften, Karl-Franzens-Universitaet, 8010 Graz, Austria, Robugen GmbH, Alleenstrasse 22, 73730 Esslingen, Germany, and Fachbereich Pharmazie, Martin-Luther-Universitaet, 06120 Halle, Germany

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Chamazulene carboxylic acid (**1**) is a natural profen with anti-inflammatory activity and a degradation product of proazulenic sesquiterpene lactones, e.g., matricin. Both **1** and proazulenes occur in chamomile (*Matricaria recutita*), yarrow (*Achillea millefolium*), and a few other *Asteraceae* species. It was isolated in improved yields, characterized physicochemically, and found to be an inhibitor of cyclooxygenase-2, but not of cyclooxygenase-1. It had anti-inflammatory activity in several animal models with local and systemic application. When human volunteers were given matricin orally, plasma levels of **1** were found to be in the micromolar range. Matricin was converted to **1** in artificial gastric fluid, but not in artificial intestinal fluid. Matricin and the yarrow proazulenes are proposed to be anti-inflammatory through conversion to **1**. Intriguingly, the biological activity of the natural compound **1** was found because of its similarity to fully synthetic drug substances. This is the reverse process of the common lead function of natural compounds in drug discovery.

Chamazulene carboxylic acid (**1**) was first isolated from yarrow (*Achillea millefolium* s.l.) in 1953,[1] many years before the development of the fully synthetic anti-inflammatory, analgetic, and antipyretic profens, e.g., ibuprofen (**2**) and naproxen (**3**). Its structural resemblance to these (by now) very common drug substances was overlooked, although yarrow and chamomile (*Matricaria recutita* L.) have been in use as antiphlogistic agents for ages.[2]

It is still a matter of conjecture which constituents are mainly responsible for the effects of preparations of these medicinal plants.[3] The azulene **1** is formed in flowers[4] or during extraction via one hydrolytic and three elimination steps from proazulenic sesquiterpene lactones, e.g., matricin (**4**) and the *Achillea* proazulenes[5] 8α-acetoxyartabsin (achillicin) (**5a**), 8α-angeloxyartabsin (**5b**), 8α-tigloxyartabsin (**5c**), and 8-deacetyl-4-epimatricin (**5d**) (Chart 1). On heating, **1** is decarboxylated to give another plant-derived azulene, chamazulene (**6**), which is responsible for the blue or greenish color of chamomile flower distillate.

The role of the proazulenes and azulenes for the net anti-inflammatory effect of chamomile and yarrow preparations is not clear, and our discovery of the activity of **1** may shed light on the various findings published in the past decades.

Even before the constitutions of **1**, **4**, and **6** were elucidated, the conspicuous change in color of extracts on heating or acidification had indicated the presence of proazulenes and azulenes. Several papers postulated that the proazulene(s) had no antiphlogistic effect. One reason for this assumption was that chamomile extracts were effective only after heating with concurrent formation of azulene(s).[6] Later it was shown that matricin was converted into the azulenes **1** and **6**. This is a fast reaction in acidic media and starts with the hydrolysis of the acetic acid ester moiety to give deacetyl matricin (**7**).[7] As for the mechanism of anti-inflammatory action, it was agreed that inhibition of enzymes of the arachidonic acid cascade was involved. Even then the structural resemblance of **1** to arachidonic acid and arylpropionic acid derivatives (profens) was not noticed. Matricin (**4**) had no inhibitory effect on the formation of cyclooxygenase (COX) and lipoxygenase (LOX) metabolites and only weakly suppressed peroxidation.[8] The proazulene was shown to be a precursor of the active substance or substances, presumably chamazulene (**6**). However, in vivo tests proved **4** to have greater activity than its degradation product **6**. With **4** being inactive in vitro and after topical application,[9] it is obvious that **4** is converted into an active metabolite. Several sesquiterpene lactones are anti-inflammatory through interference with chemokines and transcription factors, but matricin lacks an α,β-unsaturated lactone or ketone moiety essential for this mechanism of action.[10]

The pharmacokinetics of ^{14}C-labeled matricin (**4**) was investigated.[11] After oral application to mice, only very low concentrations of intact ^{14}C-**4** were found in the digestive tract, blood, and urine. In the organs, somewhat higher concentrations were found of **6** and other metabolites of low polarity, whose anti-inflammatory activity was not determined.

We undertook the investigation of the medicinal chemical properties and activity of chamazulene carboxylic acid (**1**), the primary azulene formed from **4**, considering it to be the most likely candidate for the elusive active metabolite of the proazulenic sesquiterpene lactones.

Results and Discussion

We isolated **1** from *Matricaria recutita* L., *Achillea millefolium* s.l., and *Artemisia arborescens* L. Based on the original procedure, the yield of **1** was improved. This was partly due to the proazulene rich *Achillea* cultivar we used, *Achillea collina* BECKER 'Proa'.[12] On the basis of the proazulene content in the flower heads, a yield of 80% was achieved. The azulene **1** is more stable than expected. In neutral or basic solution, it does not lose carbon dioxide; neither do salts of **1**. However, after a few days the blue solutions turned greenish because of oxidation of the azulene system to unidentified polymers. Under exclusion of oxygen, both the solutions and salts are stable for months. In organic solvents, however, decarboxylation is fast, and **6** is formed within a couple of hours. The rate of decarboxylation was followed in deuterium oxide and two buffer

* To whom correspondence should be addressed. Tel: +49-(0)345-5525175. Fax +49-(0)345-5527027. E-mail: peter.imming@pharmazie.uni-halle.de.

[†] Institut fuer Pharmazeutische Chemie, Philipps-Universitaet. These authors contributed equally to this work.

[‡] Zentrum fuer Kinder- und Jugendmedizin, Philipps-Universitaet.

[§] Martin-Luther-Universitaet.

[⊥] Heinrich-Heine-Universitaet.

[∥] Karl-Franzens-Universitaet.

[▽] Robugen GmbH.

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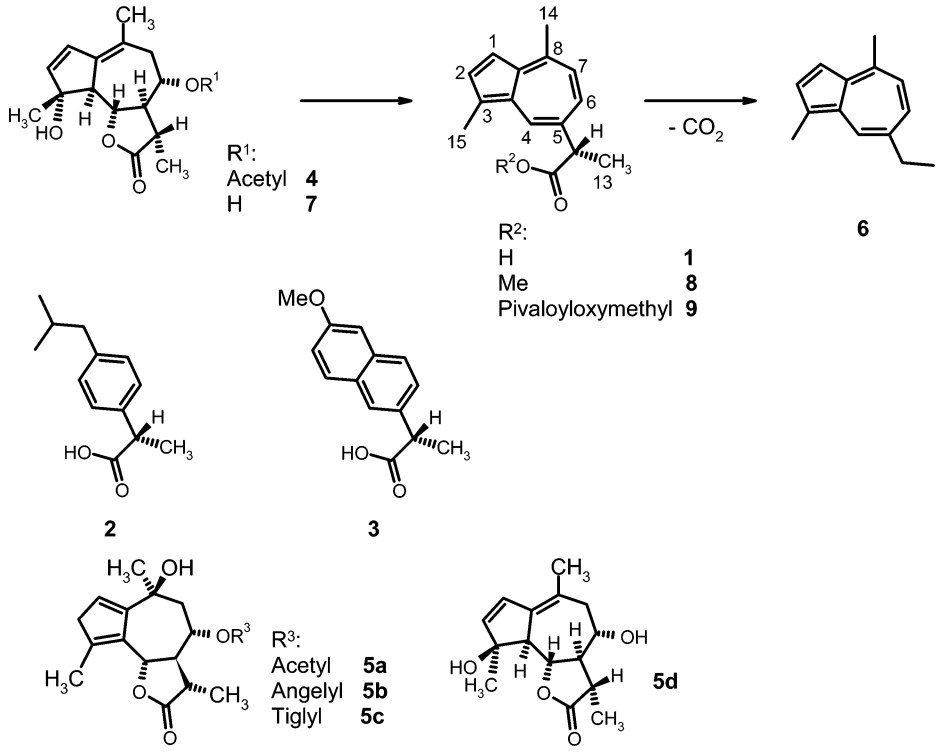
Chart 1



solutions of the European Pharmacopoeia (pH 7.5 and 2.0), all with normal oxygen content, by ^{1}H NMR (data not shown). On the basis of first-order kinetics, the rate constants k_1 were calculated to be 1.70×10^{-3} (D_2O, pH 7.0), 1.78×10^{-3} (pH 7.5), and 11.9×10^{-3} L mol^{-1} s^{-1} (pH 2.0).

Compound **1** was further characterized by determining its pK_a and log D (pH 7.4) values to be 4.14 and 0.79. This is similar to ibuprofen (5.20 and 1.07) and flurbiprofen (4.60 and 0.85),[13] so **1** should have the same excellent oral bioavailability.

The cyclooxygenases COX-1 and COX-2 are key enzymes of the arachidonic acid cascade.[14] Their inhibition by profens and other nonsteroidal anti-inflammatory compounds (NSAIDs) is thought to cause the effects of NSAIDs.[15] Structurally, **1** is a natural profen. Its stereogenic center has an *S*-configuration, the same configuration of the COX-inhibitory eutomers of profens.[16] In an in vitro assay[17] with isolated COX-1, **1** showed almost no inhibition (17.4 ± 6.2% at 50 μM, Table 1). In contrast, **1** did inhibit isolated COX-2 enzyme: 43.5 ± 4.0% at 50 μM. This is comparable to the marketed COX-2 inhibitor nimesulide, which inhibited COX-1 by 0% and COX-2 by 50 ± 3.5% at 50 μM in the same assay.

We tested **1** for topical activity in two mouse models.[18] In a model of allergic contact dermatitis, the ear lobes of mice were sensitized against 4-ethoxymethylene-2-phenyl-4*H*-5-oxazolone. For **1**, a low inhibitory effect of 23% was observed in the ear lobe edema, while *S*-naproxen showed 59% inhibition (data not shown). This result was not surprising, as chamomile extracts, preparations, and constituents have never shown activity against allergic inflammations.[2]

Application of phorbol myristyl acetate (PMA) provokes an acute inflammation (edema) of the ear lobe. In this model **1** had an inhibitory effect of 38% at a dose of 10^{-6} mol per ear after 6 h. This is almost equipotent to naproxen, which showed 45% inhibition under the same conditions.

The systemic activity of **1** was tested in the carrageenin-induced rat paw edema.[19] One dose level of 300 mg/kg was examined. One hour after administration, carrageenin was administered via subplantar injection into the left paw. The untreated right paw served as a control. Compound **1** has a slight inhibitory effect 1 h after carrageenin treatment (Table 1). The methyl and pivaloyl esters **8** and **9** were prepared as prodrugs of **1**. Supposedly due to rapid biotransformation, almost no reduction of paw volume was observed 3 h after carrageenin treatment.

Table 1. Anti-inflammatory Activity of **1**, **8**, and **9** in Comparison to Standard Compounds[a]

compound	dosage	test system or animal model	% inhibition, mean ± SEM, $n = 2$
1	50 μM	COX-1[b]	17.4 ± 6.2
1	50 μM	COX-2[c]	43.5 ± 4.0
NIM	50 μM	COX-2[c]	50.0 ± 3.5
1	0.1 μmol/ear	MEE	12 after 6 h
			19[d] after 24 h
			35[d] after 48 h
1	1.0 μmol/ear	MEE	38[d] after 6 h
			14 after 24 h
			24[d] after 48 h
NAP	0.1 μmol/ear	MEE	28[d] after 6 h
			21[d] after 24 h
			18 after 48 h
NAP	1.0 μmol/ear	MEE	45[d] after 6 h
			36[d] after 24 h
			25[d] after 48 h
1	300 mg/kg	RPE	17.4 ± 1.0 after 1 h
8	300 mg/kg	RPE	22.8 ± 3.2 after 1 h
9	300 mg/kg	RPE	34.8 ± 3.2 after 1 h
ASA	600 mg/kg	RPE	92.4 ± 5.9 after 1 h
ASA	600 mg/kg	RPE	77.3 ± 4.5 after 3 h

[a] Abbreviations: SEM, standard error of the mean. NIM: nimesulide, NAP: *S*-naproxen, ASA: acetylsalicylic acid. MEE: acute mouse ear edema provoked by phorbol myristyl palmitate, RPE: acute rat paw edema provoked by carrageenin. [b] COX-1 from ram seminal vesicles. [c] COX-2 from sheep placental cotyledons. [d] Significant against untreated controls with $p < 0.05$ (*t*-test).

At this stage, we had established a procedure to isolate the azulene **1** in gram quantities, had ascertained by determining its log D and pK_a that it would be taken up orally, and discovered it to be a COX-2 inhibitor with some topical and systemic anti-inflammatory activity in animal models. Because of its insufficient stability, the natural precursor proazulenes were considered to be suitable prodrugs of **1** in the form of plant extracts. For a bioavailability study with human volunteers, gram quantities of

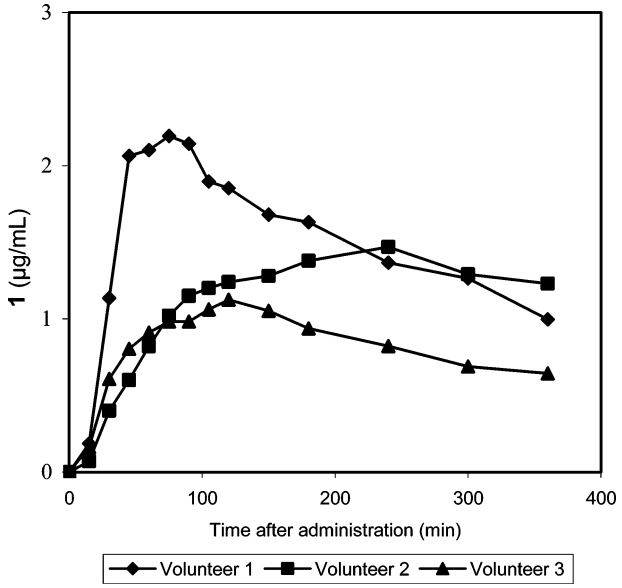


Figure 1. Plasma levels of **1** after oral administration of 500 mg of **4** to three human volunteers.

matricin (**4**) were required. Whereas *A. collina* had been the best source of **1**, the presence of four proazulenes in *Achillea* made us turn to *Matricaria recutita*. Good matricin isolation procedures have been reported,[7e] but we had to improve the scaling up because such large amounts had never been isolated. The extraction procedure is detailed in the Experimental Section. Several high-quality chamomile cultivars were assayed for matricin content by quantitative TLC scanning (data not shown). With slight variations depending on the year of harvest, the cultivar 'Mabamille' had a matricin content of approximately 1.0 mg/g dried flower heads, 'Kirsch', approximately 1.2, and 'Manzana' approximately 1.8 mg/g. 'Manzana' was used for the isolation of **4**.

On the basis of the assumption that **4** is a prodrug of **1**, it had to be decided whether to give it in a formulation resistant to gastric fluid. For this reason, it was analyzed whether **1** was converted more quickly in artificial gastric or intestinal fluid (data not shown). The artificial body fluids were prepared according to the procedure of the United States Pharmacopeia.[20] During the isolation of **1**, basic conditions at slightly elevated temperatures were needed to induce the formation of the azulene system. In artificial gastric fluid, after 30 min at 37 °C no matricin was detectable, but 50% of it was transformed to **1**. There was no chamazulene **6** detectable. In contrast, matricin was not degraded in artificial intestinal fluid after 60 min. These results indicated that matricin should be a good precursor for **1** after uncoated oral application.

An HPLC assay was established for the determination of **1** in plasma. It was based on the single extraction of **1** from acidified plasma with diisopropyl ether using ibuprofen as the internal standard. The calibration curves were linear over the concentration range 0.1−30 μg/mL. The intra-day and inter-day precision and accuracy were examined and documented. The limit of quantitation was 0.1 μg/mL. Details of the assay will be published elsewhere.[21]

An amount of 500 mg of **4** was administered orally as a suspension to three healthy volunteers. One volunteer swallowed 300 mg of **9** in a capsule. Blood samples were collected over a time of 24 h. The following prostaglandins were determined simultaneously by published methods[22] in urine collected over 24 h after the adminstration of **4** and **9**: PGE_2, PG-M, and 11-dehydrothromboxan B_2. Urine was collected on the day of administration of the test substances and the day before and after.

After the administration of matricin (**4**), chamazulene carboxylic acid (**1**) was again detected in plasma (Figure 1). Peak plasma concentrations of 0.9−2.2 μg/mL were observed after 75−90 min.

Table 2. Pharmacokinetic Parameters of **1** after Oral Administration of **4**

parameter		value
C_{max}	maximum peak plasma concentration	0.9−2.2 μg/mL
t_{max}	time of C_{max}	1.25−1.5 h
K_a	absorption coefficient	0.53 h^{-1}
α	hybrid constant of distribution	0.37 h^{-1}
β	elimination constant	0.17 h^{-1}
$t_{1/2}$	elimination half-time	4.0 h
V_d	distribution volume, f (diffusion coefficient) = 1	133 L
$(AUC)_{0-\infty}$	area under the curve	14.7 μg h/mL

After administration of the ester **9**, again **1** was detected in plasma, yielding a peak concentration of 1.7 μg/mL after 2.5 h and a second peak of 2.4 μg/mL after 5 h. This was followed by the comparatively slow elimination of **1**. A third peak plasma concentration of 2.9 μg/mL was observed 25 h after administration. The ester **9** could not be detected in the plasma samples. The third peak might be due to enterohepatic recycling of **1** and/or binding to fat tissue. This was not investigated, however.

The effects on the arachidonic acid cascade products were not significant. Only the renal excretion rate of PG-M was reduced to 40% after the oral administration of **4**, which demonstrated a systemic inhibition of prostaglandin synthesis.

Our data show that **4** is a prodrug of **1**. The pharmacokinetic parameters of the latter could be derived and are listed in Table 2. The elimination half-time of **1** was longer, and the apparent volume of distribution was larger than that of ibuprofen (**2**).

Conclusion. Although **1** is too unstable to become a drug in itself, its prodrugs are promising anti-inflammatory candidates, notably the genuine proazulenic sesquiterpenes. For therapeutic usage this implies that cultivars and extracts with a high proazulene content should be used (e.g., the chamomile cultivar Manzana or *A. collina* BECKER 'Proa'). Proazulenes are important for the anti-inflammatory action of the drugs and drug extracts. They could be markers for constant quality of drug batches if they were stabilized, e.g., by storage of the plant material at low temperature. They are indeed part of the active principle of *Achillea* and *Matricaria*. Future pharmacopoeia and ESCOP monographs should take this into account.

Intriguingly, the biological activity of the natural compound **1** was found because of its similarity to fully synthetic drug substances. This is reminiscent of the discovery of naturally occurring benzodiazepines[23] and is the reverse process of the common lead function of natural compounds in drug discovery. For instance, anti-inflammatory salicylates from willows were the template for aspirin, whereas conversely, the development of profens threw new light on anti-inflammatory medicinal plants, as shown in this paper. The antiphlogistic activities of chamomile and yarrow appear to be partly due to the conversion of guaianolides to the natural profen **1** in vivo, especially under acidic conditions (stomach, inflamed tissue).

Other prospective profen analogues from plants are currently under investigation. In a wider perspective, there may be more plant constituents awaiting discovery through comparison with synthetic drug moieties, especially if one takes possible biotransformation products into account.

Experimental Section

General Experimental Procedures. Melting points were determined on a Leitz HM-LUX melting point apparatus and are uncorrected. Mass spectra were measured with a double focusing Vacuum Generators VG 7070H at 70 eV; high-resolution mass spectra (HRMS), with a Micromass VG AutoSpec. IR spectra were recorded with a Nicolet FT-IR spectrometer 510 P. ^1H and ^{13}C NMR spectra were measured on a JEOL GX-400 (399.79 MHz for ^1H and 100.50 MHz for ^{13}C) and JEOL Eclipse+ 500 (500.00 MHz for ^1H and 125.65 MHz for ^{13}C) at

25 °C. Chemical shift values are expressed as ppm downfield from TMS. Combustion analyses were performed with a CH-analyzer Dr. Salzer (Labormatic/Wösthoff) and a CHN-autoanalyzer 185 (Hewlett-Packard; determination of N only). Measurements of pH were done with a CG822 pH-meter (Schott, Mainz, Germany) with two-point calibration using buffer solutions of pH 4.00, 7.00, and 9.00 (Merck, Darmstadt, Germany).

Plant Material. *A. collina* BECKER 'Proa' and *M. recutita* L. 'Manzana' were from controlled cultures of Martin Bauer GmbH & Co. KG, Vestenbergsgreuth, Germany.

Extraction of (*S*)-2-(α,3,8-Trimethylazulen-5-yl)acetic Acid 1 (chamazulene carboxylic acid). A 250 g amount of *M. herba* (flower heads and top 10−20 cm of stems of *A. collina* BECKER 'Proa') was extracted with $CHCl_3$ (2 L) for 1 h. The filtrate was reduced to a green sticky residue by evaporation under reduced pressure at 40 °C. The residue was taken up with 30 mL of 1 M ethanolic NaOH and heated to 45 °C for 20 min. The mixture was diluted with 150 mL of H_2O and washed three times with 200 mL of *n*-hexane each, and the aqueous phase was acidified to pH 3−4 with 10% citric acid solution. The product was extracted with pentane (200 mL, twice), and the pentane extracts were dried over $MgSO_4$, filtered, and evaporated under reduced presure at 40 °C. Column chromatography or preparative thin-layer chromatography (R_f, 0.4) eluting with *n*-hexane/EtOAc/MeOH (65:30:5) yielded a dark blue oil: 0.84 g (0.33%); UV (1.2 mg % in MeCN/H_2O, 4:6) λ_{max} (log ϵ) 217 nm (4.0), 244 nm (4.2), 285 nm (4.5); IR (neat) ν_{max} 3359, 2975, 2928, 2257, 1926, 1700, 1457, 1381, 1330, 1275, 1090, 1050 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.22−8.21 (1H, d, $^4J_{4,6}$ = 1.8 Hz, H-4), 7.64−7.63 (1H, d, $^3J_{1,2}$ = 3.7 Hz, H-2), 7.44−7.43 (1H, dd, $^3J_{6,7}$ = 10.5, $^4J_{4,6}$ = 1.8 Hz, H-6), 7.28−7.27 (1H, d, $^3J_{1,2}$ = 3.7 Hz, H-1), 7.00−6.98 (1H, d, $^3J_{6,7}$ = 10.5 Hz, H-7), 3.88−3.85 (1H, q, $^3J_{11,13}$ = 7.2 Hz, H-11), 2.82 (3H, s, H-14), 2.64 (3H, s, H-15), 1.62−1.60 (3H, d, $^3J_{11,13}$ = 7.2 Hz, H-13); ^{13}C NMR ($CDCl_3$, 100.5 MHz) δ 179.21, 145.41, 137.34, 136.71, 136.07, 135.59, 133.74, 130.79, 126.85, 124.95, 114.11, 48.75, 24.07, 18.97, 12.87; EIMS *m*/*z* 229 [M^+ + 1] (22%), 228 [M^+] (100), 183 [$M^+ - CO_2H$] (99), 168 [$M^+ - CH_3CO_2H$] (60); HRMS [M^+] $C_{15}H_{16}O_2$ calc 228.1150, found 228.1158; $C_{15}H_{16}O_2$ (228.28) calc C 78.97, H 7.06, found C 78.29, H 7.05.

Determination of the Acidity Constant and Distribution Coefficient of 1. pK_a and log D were determined using a Sirius PCA 101 pK_a and log P analyzer.

Methyl (*S*)-2-(α,3,8-Trimethylazulen-5-yl)acetate 8. 1 (0.42 g, 1.83 mmol) was dissolved in 50 mL of MeOH/benzene, 1:4. A 2.0 M solution of trimethylsilyldiazomethane in *n*-hexane (1.5 mL, 3.0 mmol) was added slowly at room temperature, and the mixture was stirred for 1 h. After evaporation of the solvent, the residue was purified by column chromatography eluting with *n*-hexane/EtOAc, 85:15, yielding 0.290 g (0.65%) of a dark blue oil: IR (neat) ν_{max} 3424, 3155, 2360, 2254, 1734, 1457, 1386, 1205, 1170, 1096 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.21−8.20 (1H, d, $^4J_{4,6}$ = 1.8 Hz, H-4), 7.64−7.63 (1H, d, $^3J_{1,2}$ = 3.7 Hz, H-2), 7.44−7.43 (1H, dd, $^3J_{6,7}$ = 10.5, $^4J_{4,6}$ = 1.8 Hz, H-6), 7.28−7.27 (1H, d, $^3J_{1,2}$ = 3.7 Hz, H-1), 7.00−6.98 (1H, d, $^3J_{6,7}$ = 10.5 Hz, H-7), 3.88−3.85 (1H, q, $^3J_{11,13}$ = 7.2 Hz, H-11), 3.65 (3H, s, OCH_3), 2.83 (3H, s, H-14), 2.66 (3H, s, H-15), 1.61−1.60 (3H, d, $^3J_{11,13}$ = 7.2 Hz, H-13); ^{13}C NMR ($CDCl_3$, 100.5 MHz) δ 175.26, 145.31, 137.36, 136.65, 136.07, 135.71, 133.77, 131.59, 126.69, 125.00, 113.96, 52.12, 48.92, 24.09, 19.42, 12.90; EIMS *m*/*z* 242 [M^+] (100%), 183 [$M^+ - COOCH_3$] (87), 243 [M^+ + 1] (82), 168 (58), 155 (57); $C_{16}H_{18}O_2$ (242.31) calc C 79.33, H 7.48, found C 79.48, H 7.41.

2,2-Dimethylpropionyloxymethyl (*S*)-2-(α,3,8-Trimethylazulene-5-yl)acetate 9 (pivaloyloxymethyl ester of 1). Compound **1** (0.37 g, 1.6 mmol) was dissolved in 3 mL of 2-propanone. Chloromethyl pivalate (0.23 mL, 1.6 mmol) and Et_3N (1.3 mL, 3.5 mmol) were added. After stirring at room temperature for 24 h, the solvent was removed by evaporation under reduced pressure and the residue taken up with a 1:1 mixture of H_2O and EtOAc (100 mL). The organic phase was dried over K_2CO_3 and filtered, and the solvent was evaporated. Column chromatography eluting with *n*-hexane/*tert*-butylmethyl ether, 8:2, yielded 0.37 g (37%) of a dark blue oil: IR (neat) ν_{max} 3445, 3069, 2357, 1756, 1700, 1558, 1457, 1117, 1078, 990 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.20−8.19 (1H, d, $^4J_{4,6}$ = 2.0 Hz, H-4), 7.65−7.64 (1H, d, $^3J_{1,2}$ = 3.7 Hz, H-2), 7.43−7.40 (1H, dd, $^3J_{6,7}$ = 10.5, $^4J_{4,6}$ = 2.0 Hz, H-6), 7.23−7.22 (1H, d, $^3J_{1,2}$ = 3.7 Hz, H-1), 7.04−6.97 (1H, d, $^3J_{6,7}$ = 10.5 Hz, H-7), 5.75−5.71 (2H, 2 d, ^2J = 5.5 Hz, O−CH_2−O), 3.89−3.80 (1H, q, $^3J_{13,11}$ = 7.1 Hz, H-11), 2.83 (3H, s, H-14), 2.62 (3H, s, H-15), 1.62−1.61 (3H, d, $^3J_{13,11}$ = 7.1 Hz, H-13), 1.07 (9H, s, H-3); ^{13}C NMR ($CDCl_3$, 125.65 MHz) δ 176.89, 173.36, 145.28, 137.32, 136.7, 135.65, 135.49, 133.63, 130.50, 126.87, 124.89, 114.10, 79.35, 48.71, 38.52, 26.83, 24.00, 19.38, 12.84; EIMS *m*/*z* 343 [M^+ + 1] (34), 342 [M^+] (100), 183 [$M^+ - (CH_3)_3C - C(O)O - CH_2 - OC(O)$] (68), 169 [$M^+ - (CH_3)_3C - C(O)O - CH_2 - OC(O) - CH_3$] (9); $C_{21}H_{26}O_4$ (342.43) calc C 73.60, H 7.60, found C 73.15, H 7.57.

Extraction of (−)-(3*S,3a*R**,4*S**,9*R**,9a*S**,9b*S**)-4-acetoxy-2,3,-3a,4,5,9,9a,9b-octahydro-9-hydroxy-3,6,9-trimethylazuleno[4.5*b*]-furan-2-one 4 (matricin).** A 300 g sample of *M. flos* (flower heads of *M. recutita* L. 'Manzana') with a matricin content of approximately 1.8 mg/g (determined by quantitative TLC; data not shown) was steeped in $CHCl_3$ (2.5 L) and kept standing for 1.5 h with occasional stirring. After suction filtration, the filtrate (approximately 1.5 L) was evaporated under reduced pressure, keeping the water bath below 30 °C. This gave 13 g of a brown-yellow mass. It was heated to 30 °C and poured into 400 mL of petroleum ether at approximately 40 °C with stirring. The resulting brown-yellow precipitate was removed by suction filtration, and the filtrate again evaporated to give 10.5 g of a brown-yellow residue. MeOH (50 mL) was added, and the suspension kept in an ultrasonic bath for 20 min. The precipitated wax was removed by filtration through a layer (5 mm) of neutral aluminum oxide in a glass filter funnel. Evaporation of the filtrate yielded 5 g of a brown-yellow residue, which was chromatographed (column length, 27 cm, diameter, 3 cm) through silica gel (0.063−0.2 mm, approximately 200 g). Elution started with $CHCl_3$/*tert*-butylmethyl ether, 5:1, and continued with pure *tert*-butylmethyl ether as soon as matricin elution began (TLC monitoring). A total of approximately 500 mL of fractions yielded 0.65 g of residue. The residues from four to five extractions were combined and dissolved in 300 mL of petroleum ether at 30−40 °C. The turbid yellowish solution was extracted with 150 mL of a 2% (w/v) $KHCO_3$ solution of pH 8.5. The extraction and shaking had to be done gently in order to avoid the formation of a stable emulsion. The clear light yellow aqueous phase was separated and extracted three times with 50 mL of Et_2O each. The combined ether phases were dried over 8 g of anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure at room temperature. The extraction of the petroleum ether with $KHCO_3$ solution and re-extraction with ether was repeated 12 times, reusing the $KHCO_3$ solution.

On concentrating the dried Et_2O extracts, yellowish matricin crystallized. After recrystallization from Et_2O, 0.29 g of pale yellow crystals was obtained. This was dissolved in a small amount of $CHCl_3$ and chromatographed through 100 g of neutral aluminum oxide (activity grade IV), eluting with CH_2Cl_2/MeOH, 99:1. By TLC monitoring the fractions that contained matricin were identified, pooled, and evaporated. After recrystallization from Et_2O, 0.26 g of white crystals was obtained and stored at −20 °C: mp (dec) 158−160 °C; UV (0.7 mg % in CH_2Cl_2) λ_{max} (log ϵ) 245 nm (4.3); IR (KBr) ν_{max} 3200, 2900, 1750, 1719, 1260, 1090, 1050, 1000, 980 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.32−6.31 (1H, d, $^3J_{2,3}$ = 5.7 Hz, H-2), 5.94−5.93 (1H, d, $^3J_{2,3}$ = 5.7 Hz, H-3), 4.96−4.94 (1H, dt, $^3J_{7,8}$ = 11.5, $^3J_{8,9a}$ = 10.3, $^3J_{8,9e}$ = 3.6 Hz, H-8), 4.11−4.07 (1H, t, $^3J_{5,6}$ = 11.0 Hz, H-6) 2.92−2.90 (1H, dquint, $^3J_{5,6}$ = 11.0, $^5J_{5,9a}$ = 2.1, $^5J_{5,14}$ = 2 Hz, H-5), 2.57−2.56 (1H, dquart, $^3J_{11,7}$ = 11.5, $^3J_{11,13}$ = 6.9 Hz, H-11), 2.47−2.45 (1H, br dd, $^2J_{9a,9e}$ = 15.8, $^3J_{8,9a}$ = 10.3, $^5J_{5,9a}$ = 2 Hz, H-9a), 2.41−2.40 (1H, dd, $^2J_{9a,9e}$ = 15.8, $^3J_{8,9e}$ = 10.3 Hz, H-9e), 2.22−2.02 (1H, dt, $^3J_{7,8}$ = 11.5, $^3J_{11,7}$ = 11.5 Hz, H-7), 2.11 (1H, s, H-17), 1.85 (3H, t, $^5J_{14,5}$ = 2 Hz, H-14), 1.41 (3H, s, H-15), 1.34−1.32 (3H, d, $^3J_{11,13}$ = 6.9 Hz, H-13); ^{13}C NMR ($CDCl_3$, 125.65 MHz; assignments through heteronuclear correlation experiments) δ 177.57 (C-12), 169.94 (C-16), 140.45 (C-3), 136.61 (C-1), 129.95 (C-2), 123.80 (C-10), 84.18 (C-4), 79.32 (C-6), 71.75 (C-8), 58.80 (C-5), 56.58 (C-7), 42.43 (C-9), 40.37 (C-11), 25.16 (C-15), 23.51 (C-14), 21.13 (C-17), 15.40 (C-13); EIMS *m*/*z* 228 (100%), 288 [$M^+ - H_2O$] (64), 185 (63), 229 (39), 306 [M^+] (1); HRMS [M^+] $C_{17}H_{22}O_5$ calc 288.1362, found 288.1348 [$M^+ - H_2O$]; $C_{17}H_{22}O_5$ (306.36) calc C 66.65, H 7.24, found C 66.45, H 7.10.

Inhibition of Cyclooxygenase-1 and -2. It was determined for **1** and nimesulide according to a published procedure.[17]

Topical Anti-inflammatory Activity: Inhibition of Mouse Ear Lobe Edema by 1. The inhibition of the allergic and acute edema of mice ear lobes was determined following the published protocols.[18]

Systemic Anti-inflammatory Activity: Inhibition of Rat Paw Edema by 1. The inhibition of the rat paw edema induced by subplantar injection of carrageenin was determined by the standard model.[19]

HPLC Assay for the Determination of 1 in Plasma. Sample preparation was performed by liquid−liquid extraction using diisopropyl ether as extracting solvent after acidification with 0.01 M H_3PO_4. The recovery of CCA from serum ranged from 93 to 112%. The coefficients of variation were lower than 10%. The recovery of ibuprofen was 96% at the concentration used in the assay (30 μg/mL). The calibration curves were linear over the concentration range 0.1−30 μg/mL. The calibration graph showed excellent linear relationships with a mean correlation coefficient r^2 of 0.9994. The mean regression line was $y = 0.1198(+0.0024)x - 0.0058(+0.013)$. The intra- and inter-day precision (%CV) was determined by repeated ($n = 6$) analysis of four spiked serum samples containing low, medium, and high concentrations of CCA. %CV was less than 11% over a wide range of concentrations. The within- and between-day relative errors assessed during the replicate analyses varied between 10.0 and +7.0%. The limit of quantitation (LOQ) of this assay was 0.1 μg/mL in serum. The limit of quantitation was 0.1 μg/mL. Details of the assay will be published elsewhere.

Bioavailability: Oral Application of Matricin 4 and Resulting Plasma Levels of 1. With the approval of the Ethics Commision of the Universitätsklinikum Marburg, Germany, three healthy volunteers aged 25−30 swallowed an aqueous suspension of 500 mg of **4** on an empty stomach. The volunteers had refrained from taking any drugs a week before. On the basis of our experiments on the decomposition of **4** in artificial gastric fluid, the dose of **4** was calculated to yield a dose of **1** equivalent to 200 mg of ibuprofen. Blood samples were taken before and after the administration at regular intervals, as shown in Figure 1.

Bioavailability: Oral Application of the Pivaloyl Ester 9 and Resulting Plasma Levels of 1. With the approval of the Ethics Commision of the Universitätsklinikum Marburg, Germany, one male healthy volunteer aged 28 swallowed 300 mg of **9** in an uncoated gelatine capsule on an empty stomach. The volunteer had refrained from taking any drugs a week before. The dose of **9** was calculated after ester hydrolysis to yield a dose of **1** equivalent to 200 mg of ibuprofen. Blood samples were taken before and after the administration at the same intervals as in the previous experiment.

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