cyclo[4.2.0]oct-2-ene-2-carboxylic Acid (8). To a stirred solution of 7 (25.7 g, 0.047 mol) and anisole (25 mL) in dry CH_2Cl_2 (250 mL) was added trifluoroacetic acid (250 mL) under an argon atmosphere. Stirring was continued at room temperature for 3 h, and the reaction was then evaporated in vacuo. The resulting oily residue was triturated with AcOEt (200 mL) for 20 min to induce precipitation. Anhydrous ether (650 mL) was added to this suspension, and the mixture were stirred for a further 20 min. Due to the hygroscopic nature of the product, the precipitate was filtered under a nitrogen atmosphere to afford 20.25 g of 8 (76%): ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3 H, CH_3), 2.31 (s, 3 H, CH_3), 3.65, 3.76 (AB, 2 H, J_{gem} = 18 Hz, SCH_2), 4.91 (d, 1 H, J = 5.0 Hz, CH), 5.07 (d, 1 H, J = 5.0 Hz, CH), 4.96, 5.29 (AB, 2 H, J_{gem} = 12.8 Hz, OCH_2), 7.46 (d, 1 H, J = 8.4 Hz, Ar), 7.85 (d, 1 H, J = 1.7 Hz, Ar), 7.93 (dd, 1 H, J = 1.7, 8.4 Hz, Ar); IR (KBr) 1800, 1775, 1720, 1680 cm^{-1}; HR-MS m/z 473.0675 (M + Na$^+$, $C_{19}H_{18}N_2O_9SNa$ requires 473.0631).

[6*R*,7*R*(*Z*)]-7-[[[(2-Amino-2-oxoethoxy)imino](2-amino-4-thiazolyl)acetyl]amino]-3-[[(3,4-dihydroxybenzoyl)oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Sodium Salt (9). To the suspension of 8 (25.2 g, 0.0446 mol) in THF (390 mL) was added a solution of $NaHCO_3$ (7.3 g, 0.087 mol) in H_2O (500 mL). The resulting mixture was stirred until a clear solution occurred. To this was added *S*-(2-benzothiazolyl) (2-amino-4-thiazolyl)-(*Z*)-[(2-amino-2-oxoethoxy)imino]acetate (23.3 g, 0.059 mol), and the reaction was stirred at room temperature for 5 h. The resulting solution was poured into a mixture of AcOEt (1.9 L) and H_2O (0.4 L) containing $NaHCO_3$ (3.7 g) and then extracted thoroughly. The aqueous layer, after separation and filtration through Celite, was further washed with AcOEt (1.9 L). The aqueous solution was then treated with MeOH (95.6 mL) and $NaHCO_3$ (19.7 g) at room temperature for 2 h. The organic solvents were removed under reduced pressure, and the reaction was acidified with 1.0 N HCl to pH 2.8 while cooling in an ice bath. The precipitate which formed was filtered and dried in vacuo to give 26.02 g of the free acid of 9.

To a 10% solution of H_2O in acetone (800 mL) was added 26.02 g (0.044 mol) of the free acid of 9, and the mixture was stirred for 30 min until most of the sample dissolved. The solution was diluted with acetone to a volume of 3 L and filtered. To the filtrate was slowly added a solution of sodium 2-ethylhexanoate (8.5 g, 0.051 mol) in acetone (1 L) with stirring over a period of 2 h. The precipitate was filtered and crystallized from H_2O (150 mL) to give 17.5 g of 9 (65%): ^{1}H NMR (DMSO-d_6) δ 3.30, 3.57 (AB, 2 H, J_{gem} = 17.1 Hz, SCH_2), 4.41 (s, 2 H, OCH_2), 4.89, 5.17 (AB, 2 H, J_{gem} = 12.0 Hz, OCH_2), 5.05 (d, 1 H, J = 4.7 Hz, CH), 5.64 (dd, 1 H, J = 4.7, 8.0 Hz, CH), 6.8 (d, 1 H, J = 8.2 Hz, Ar), 6.85 (s, 1 H, Ar), 7.12 (s, 1 H, NH_2), 7.30 (br s, 3 H, NH_2 and Ar), 7.36 (s, 1 H, Ar), 7.50 (s, 1 H, NH_2), 9.75 (d, 1 H, J = 8.0 Hz, NH), 9.46 (br, 1 H, OH), 9.91 (br, 1 H, OH); IR (KBr) 3300, 1755, 1708, 1680, 1655 cm^{-1}; HR-MS m/z 615.0599 (M + H$^+$, $C_{22}H_{20}N_6O_{10}S_2Na$ requires 615.0580).

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Registry No. 6, 37051-06-2; 7, 139290-97-4; 8, 141344-49-2; 9, 122005-95-2; 9 free acid, 122005-44-1; 10, 141344-50-5; 11, 141344-51-6; 12, 141344-52-7; 13, 141344-53-8; 3,4-bis(acetyloxy)benzoic acid, 58534-64-8; hexanoic acid, 142-62-1; cyclohexanecarboxylic acid, 98-89-5; phenylacetic acid, 103-82-2; oxolinic acid, 14698-29-4; *N*-methyl-2-fluoropyridinium tosylate, 58086-67-2; *S*-(2-benzothiazolyl) (2-amino-4-thiazolyl)-(*Z*)-[(2-amino-2-oxoethoxy)imino]thioacetate, 89876-15-3.

Supplementary Material Available: ^{1}H NMR spectra for compounds 7–13 (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

The Biogenetic Origin of the Carbon Skeleton and the Oxygen Atoms of Elaiophylin, a Symmetric Macrodiolide Antibiotic

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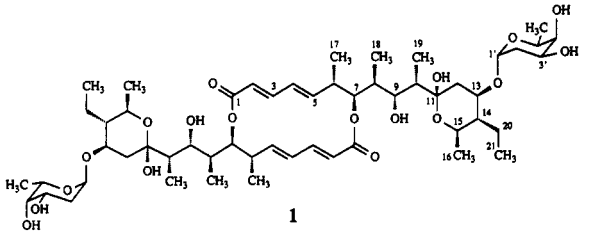
Elaiophylin (1),[1-4] a 16-membered macrodiolide antibiotic exhibiting C_2 symmetry was originally isolated from *Streptomyces melanosporus*, later as azalomycin B from *Streptomyces hygroscopicus* var. *azalomyceticus*, and also from *Streptomyces violaceoniger* (Tü 905).[5,6] A high-producing strain (*Streptomyces* sp., DSM 3816), which also biosynthesizes the antibiotics niphimycin and nigericin as well as two novel niphimycin analogues,[7] was detected in the course of our chemical screening program.[8] Elaiophylin exhibits antibacterial as well as in vivo anthelminthic activity. Because of its multifunctionalized polyketide aglycon moiety deriving from two identical halves, elaiophylin (1) attracted our interest in studying modern



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hypotheses for polyketide biosynthetic pathways (see also Discussion).[9-11] On the basis of detailed NMR spectroscopic analysis,[1] we present here the first results of our biosynthetic studies, namely the biogenetic assembly of elaiophylin (1) formed via a mixed biogenesis from polyketide and carbohydrate building blocks.

Experimental Section

Fermentation. The two-step fermentations of *Streptomyces* sp. (DSM 4137) were carried out using a loopful of agar slants (medium: 2% soybean meal; degreased, 2% mannitol, 1.5% agar; incubation time: 6 weeks at 30 °C to gain highest productivity) shaken in 5 × 100 mL of culture medium (2% soybean meal, degreased, 2% mannitol) in 250-mL Erlenmeyer flasks at 180 rpm at 30 °C for 3 days; i.e., the fermentation scale in each experiment was 0.5 L. An aliquot of this seed culture (10%) was used to inoculate the same medium for production (harvest after 5 days). The production time course was examined during a 50-L fermentation. Samples of 100 mL were taken every 6 h, extracted with 300 mL of ethyl acetate each, and analyzed by HPLC using a reversed-phase column (KONTRON Spherisorb 10μm, ODS RP-18, 25 cm × 4.6 mm, methanol, detection at 265 nm; retention time of 1: 3.1 min at 1 mL/min). In a typical fermentation, the production of elaiophylin (1) started 40 h after inoculation and increased by further cultivation; after ca. 100 h the concentration of 1 remained constant (maximum yield: ca. 300 mg/L).

Feeding Experiments. Feeding experiments were carried out by addition of labeled precursors in equal portions 45, 50, 55, and 60 h after inoculation (total amounts: see Table I). The feeding experiment with [1-^{13}C]-D-glucose was accomplished with replacement culture techniques because glucose is the major carbon source of the microorganism. Seventy-two hours after inoculation the culture was centrifuged (9000 rpm, 20 min). The cells were reincubated in a medium containing 10 g/L of soybean meal

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Table I. Chemical Shifts and Specific Incorporations[a] (Standardized on the C-6′ Signal Intensity) of the Proton Noise Decoupled ^{13}C NMR Resonances of Elaiophylin (1) after Feeding with Different Stable Isotope Labeled Precursors

C-atom	δ (ppm)	A	B	C	D	E	F
1	168.5	2.8	3.6	–	2.0	–	–
2	121.5	–	–	–	–	–	–
3	145.3	3.7	4.0	0.9	2.6	–	–
4	131.0	–	–	–	–	–	–
5	145.1	1.6	–	25.4	1.8	–	–
6	41.6	–	–	–	–	–	–
7	76.8	1.6	–	25.6	1.7	–	–
8	36.8	–	–	–	–	–	–
9	70.6	1.6	–	27.8	1.7	–	–
10	43.0	–	–	–	–	–	–
11	99.9	3.2	3.4	–	1.3	–	–
12	38.2	–	–	–	–	–	–
13	69.4	3.4	1.3	–	60.1	–	–
14	48.3	–	–	–	–	–	–
15	66.7	3.9	4.0	–	3.6	–	–
16	18.9	–	–	–	–	–	–
17	15.4	–	–	–	–	–	–
18	9.4	–	–	–	–	–	–
19	7.1	–	–	–	–	–	–
20	19.1	3.3	0.6	–	0.8	1.4	–
21	8.5	–	–	–	–	–	–
1′	93.7	–	–	–	–	–	3.5
2′	33.1	–	–	–	–	–	–
3′	65.9	–	–	–	–	–	–
4′	71.2	–	–	–	–	–	–
5′	67.2	–	–	–	–	–	–
6′	17.1	–	–	–	–	–	–

[a] Calculated according to Scott, Townsend et al.:[33] –, spc. incorporation ≤0.5%; A, sodium [1-^{13}C]acetate, 1 g/L; yield of 1, 278 mg/L; B, [1,3-$^{13}C_2$]malonic acid, 1 g/L; yield of 1, 320 mg/L; C, sodium [1-^{13}C]propionate, 1 g/L; yield of 1, 78 mg/L; D, sodium [1-^{13}C]butyrate, 0.5 g/L; yield of 1, 48 mg/L; E, sodium [3-^{13}C]-butyrate, 0.2 g/L; yield of 1, 244 mg/L; F, [1-^{13}C]glucose, 2.5 g/L (see text).

(degreased), 2.5g/L of unlabeled and 2.5 g/L of [1-^{13}C]glucose for 48 h.

The labeled compounds ([1-^{13}C]acetate, [1,2-$^{13}C_2$]acetate, [1,3-$^{13}C_2$]malonic acid, [1-^{13}C]propionate, [1-^{13}C]butyrate, and [1-^{13}C]glucose, 99% isotope enrichment each) were obtained from Cambridge Isotope Laboratories, CIL, Cambridge, MA); the [1-$^{13}C^{18}O_2$]acetate (99% ^{13}C, 70% ^{18}O), [1-$^{13}C^{18}O_2$]propionate (99% ^{13}C, 65% ^{18}O), and [1-$^{13}C^{18}O_2$]butyrate (99% ^{13}C, 70% ^{18}O enrichment) were synthesized[12] from the [1-^{13}C]-labeled precursors and H_2 ^{18}O (97.3% ^{18}O, obtained from Isotec Inc., Miamisburg, OH, the ^{18}O-enrichments were calculated from the ^{13}C NMR spectra. The [3-^{13}C]butyrate, 99% ^{13}C enrichment, was obtained from Aldrich Chemical Co. (Milwaukee, WI).

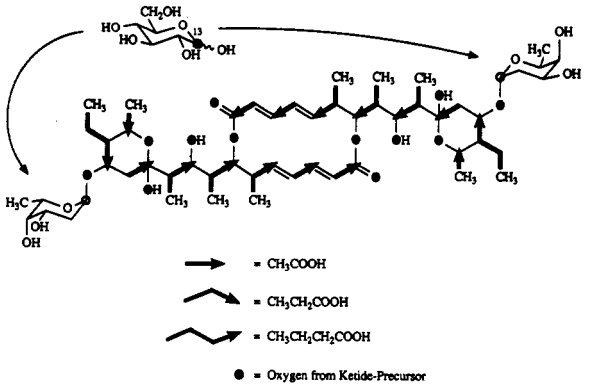

Figure 1. Biogenesis of the carbon and oxygen atoms of elaiophylin (1).

Isolation Procedure. Isolation and purification of 1 was effected by centrifugation of the culture broth (20 min, 4000 rpm) and extraction of the mycelium (3 times with 20 mL of ethyl acetate). The organic layer was evaporated to dryness, and the oily residue was chromatographed on a silica gel column (silica gel 60, <0.08 mm, Macherey & Nagel, Germany) to obtain pure colorless, amorphous 1, which showed identical physicochemical properties (MS, IR, UV, ^1H and ^{13}C NMR spectra, $[\alpha]_D$ value) as reported in the literature.[1,2,5]

NMR Experiments. ^{13}C-NMR spectra of the labeled elaiophylin samples were recorded either at 50.3, at 75.5, or at 125.7 MHz, in pyridine-d_5 each, resulting in 27 carbon signals due to the C_2 symmetry of the molecule. The signals were unequivocally assigned in earlier studies.[1]

Results and Discussion

An obvious working hypothesis for the biosynthesis of 1 envisioned a polyketide-type pathway, which led us to feed typical polyketide precursors, such as acetate, propionate, etc. The incorporations of the different labeled precursors are summarized in Table I. Therefore, with the exception of the sugar moieties, the labeling pattern pointed out that the origin of the carbon skeleton of 1 derives from the polyketide biosynthetic pathway, namely from eight acetate (malonate), six propionate, and two butyrate units, condensed in the head-to-tail fashion typical of polyketide biogenesis (Figure 1). Thus, the origin of the three methyl groups (C-17–C-19) from propionate, and not from methionine, follows the rule that C_3 units in polyketides are elaborated usually by *Actinomycetes* from propionate, whereas those biosynthesized by *Fungi* are formed from acetate and methionine.[13]

The much higher incorporation of propionate or butyrate in comparison to acetate and malonate (see Table I) has been observed previously[14] and reflects most likely the fact that the former precursors are less ubiquitious than the latter ones. An additional factor might be the increased cell membrane permeability of the more lipophilic precursors propionate and butyrate. Thus, we also observed "scrambling" of the propionate/butyrate label into the carbon atoms deriving from the carboxyl group of acetate, caused by the propionyl-CoA and butyryl-CoA catabolism to malonyl-CoA or acetate via succinyl-CoA and β-oxidation, respectively.[15-19]

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Table II. Feeding Experiments with [1-^{13}C,$^{18}O_2$]-Labeled Polyketide Precursors. The Upfield Shift of a ^{13}C Signal (at 125.7 MHz) Indicates the Direct Connection of an ^{18}O-Atom

		^{18}O-enrichment[a] and upfield shifts of ^{18}O-connected carbons		
		A	B	C
C-atom	δ (ppm)	%^{18}O/Δδ (ppm)	%^{18}O/Δδ (ppm)	%^{18}O/Δδ (ppm)
1	168.5	34.7/0.04	-	-
7	76.8	-	46.6/0.04	-
9	70.6	-	44.8/0.02	-
11	99.9	34.8/0.03	-	-
13	69.4	28.1/0.03	-	51.4/0.03
15	66.7	38.9/0.02	-	-

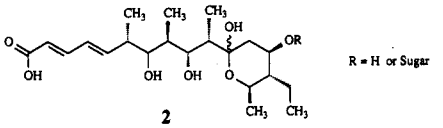
[a] Calculation: (integral of the ^{18}O-connected carbon signal/sum of the integrals of ^{16}O- and ^{18}O-connected carbon signals) × 100. A: Feeding with sodium [1-^{13}C,$^{18}O_2$]acetate, 99% ^{13}C, 70% ^{18}O; 500 mg/0.5 L. B: Feeding with sodium [1-^{13}C,$^{18}O_2$]propionate, 99% ^{13}C, 65% ^{18}O; 500 mg/0.5 L. C: Feeding with sodium [1-^{13}C,$^{18}O_2$]butyrate, 99% ^{13}C, 70% ^{18}O; 250 mg/0.5 L.

It is remarkable that also some of the butyrate-derived carbons, namely C-13 and C-20, showed a similar incorporation rate as the carbons deriving directly from acetate (C-1, C-3, C-11, C-15). In addition, the propionate-derived carbons (C-5, C-7, and C-9) were also slightly enriched from the acetate feeding. Both "scramblings" indicate a more direct formation of propionate and butyrate from acetyl-CoA than from malonyl-CoA. This is not surprising for the propionate formation via the citric acid cycle,[16] but unusual for the butyrate formation. We propose here a direct condensation of two acetyl-CoA units (e.g., catalyzed by acetyl-CoA-acetyl-transferase),[20] respectively, rather than the usual fatty acid pathway using one acetate and one malonate[16] or butyrate formation from valine.[21] This conclusion is also supported by the results of the [1,3-$^{13}C_2$]malonate feeding showing a lower incorporation into the propionate and butyrate units, respectively, than into the C_2-building blocks derived more directly from malonate (i.e., into C-1, C-3, and C-11). An indirect consequence, taken by comparison of the incorporation rates from acetate vs. malonate, indicates that C-15/C-16, and not C-20/C-21, is the polyketide starter unit of elaiophylin. Moreover, a direct "starter effect", i.e., a better incorporation of acetate into the starter unit than into the following acetate-derived C_2-units, and vice versa with malonate, could not be observed.[22] The conclusion that C-15/C-16 is the starter unit of 1 is also supported by the fact that the [1-^{13}C]butyrate feeding led to clearly higher enrichment of C-15 (through degradation of butyrate into acetate via β-oxidation) than of C-20. Remaining doubts within this "starter ambiguity" could be overcome finally by a feeding experiment with [3-^{13}C]butyrate yielding 1 with an enriched C-20, and no enrichment in C-15 (Table I). The much weaker specific incorporation resulting from the latter experiment in comparison with the [1-^{13}C]-butyrate feeding is unexpected and intriguing, and might be partially explained by the smaller amount of added butyrate, while the yield of elaiophylin (1) was approximately 5-fold (see footnote, Table I). Thus, it seems to be likely that butyrate (and also propionate), when fed in large amounts, cause a shut off of their own endogenous de novo synthesis along with a repression of the elaiophylin (1) biosynthesis (see yields of 1 in the footnote, Table I).

The ^{13}C NMR spectrum of the elaiophylin (1) sample obtained from the [1-^{13}C]glucose feeding experiment showed a significant enhancement of C-1′ proving that the 2-deoxyfucose moieties of 1 derive from glucose.

The feeding experiments with the [^{13}C, ^{18}O]-labeled precursors showed all aglycon oxygen atoms were derived from the polyketide precursor (Table II). The upfield shifts of the ^{18}O-connected carbons are of the expected magnitude.[23] C-1 and C-7 show a shift at the lower limit for a carbonyl and at the upper limit for a secondary alcohol, respectively.[23] Thus, electron delocalization between C-1, C-7 and the lactone oxygens can be assumed.

It seems likely that two identical, activated octaketide CoA-derivatives, formed as complete functionalized intermediates, were intermolecularly cyclized to the bislactone, thus forming the central 16-membered ring in 1. The highly specific incorporation of [1-^{13}C]butyrate or [1-^{13}C]propionate into 1 suggests a simple approach to prove this hypothesis via degradation of the molecule to the corresponding elaiophylinic acid (2),[24] which might



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then function as the immediate octaketide precursor of elaiophylin (1). Thus, 1 may serve as an interesting model molecule for providing further support of the current and widely accepted view of polyketide formation, that considerable functionality in partially reduced polyketides appears as a result of an assembly process resembling incomplete fatty acid biosynthesis.[10,11] This hypothesis has been supported by intact incorporation of correctly functionalized (via synthesis) di- and triketides[10] and, more recently, of a tetraketide.[25] In only one case, however, has it been accomplished with a complete parent polyketide, i.e., a pentaketide derivative,[26] but not yet with an octa- or decaketide. The chance of an intact incorporation of an octaketide precursor into 1 may be higher since the molecule has to be assembled from two identical octaketide halves at a late step of its biosynthesis. It is likely that this transformation is catalyzed by a separate enzyme following the biosynthesis of the parent polyketide by a multienzyme complex (polyketide synthase). This question in the context of the cyclization of the two 2,4-dihydroxypyrane rings and the connection of the L-2-deoxyfucose units in the course of the biosynthesis of 1 are subjects of our further investigations. The dihydroxypyrane moiety, which may be formed biosynthetically by hemiketalization at the stage of the initial triketide, plus the two following propionate units occur also in nonsymmetrical macrolides, such as the bafilomycins,[27] the con-

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canamycins,[28] and virustomycin A.[29] Despite their starting units deriving from obviously different polyketide building blocks, the stereochemistry of the pentaketide moiety in 1 is the same as that in the molecules mentioned above. Further biosynthetic studies on elaiophylin (1) may also have implications for the biosyntheses of other C_2 symmetric macrodiolides, e.g., vermiculin, conglobatin, pyrenophorin or swinholide A.[30-32]

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A Novel Synthesis of the Dibenz[*b*,*f*]oxepin Ring System: 10,11-Dihydro-11-hydroxydibenz[*b*,*f*]oxepin-10(11*H*)-one

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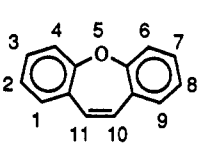
Introduction

The synthesis of dibenz[*b*,*f*]oxepin (1) was first reported in 1950 by Manske[2] and was later also synthesized by Bestmann.[3] Interestingly, the structure of pacharin (2), which was isolated from the heartwood of *Bauhinia racemosa* Lamk, has been also established as having a dibenz[*b*,*f*]oxepin skeleton.[4] In fact, derivatives of dibenz[*b*,*f*]oxepin have been found to exhibit depressant,[5] anticonvulsant,[6] analgesic,[7] antiinflammatory,[8] tranquilizing,[9] psychotropic,[10] sedative,[11] and antiestrogenic[12] properties. Our need for the identification and synthesis of new A_1 adenosine receptor ligands[13] led us to prepare functionalized dibenz[*b*,*f*]oxepins, particularly molecules that bear oxygenated functional groups at the two olefinic carbons. In this note, we report the synthesis of 10,11-dimethoxydibenz[*b*,*f*]oxepin (3) by the methylation of 10,11-dihydro-11-hydroxydibenz[*b*,*f*]oxepin-10(11*H*)-one (6). Compound 6 was itself prepared by a benzoin reaction on bis(2-formylphenyl) ether (5).



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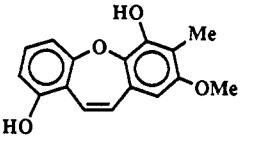

2

Results and Discussion

Diol 4[14] was oxidized by the Jones procedure[15] to give dialdehyde 5[16] in 47% yield (Scheme I). The benzoin condensation of 5 with potassium cyanide in dimethyl sulfoxide afforded benzoin 6 in 25% yield, together with a small amount of diketone 7.[17] It is worth noting that a longer reaction time produced more 7 and was therefore detrimental to the preparation of 6. Surprisingly, attempted formation of 3 from 6 by employing the conventional procedure gave only the methoxy ketone 8.[18] Our target molecule 3 was obtained as a colorless oil in 76% yield from 6 by treatment with sodium hydride and dimethyl sulfate in tetrahydrofuran.

The pharmacological profile of 3 is now under investigation.

Experimental Section

TLC plates were purchased from commercially available precoated Merck Kieselgel 60 F_{254} on aluminum. Column chromatography was carried out using Merck silica gel (70–230 mesh). All evaporations were performed under reduced pressure with a rotary evaporator. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ at 250 and 62.5 MHz, respectively. Melting points were recorded on a hot-stage microscope and are uncorrected.

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