Synthesis of carboxyalkylindolenines from 6-methyl-7-oxooctanoic acid

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Novel 3-(4-carboxybutyl)indolenines were obtained. A method for the synthesis of 6-methyl-7-oxooctanoic acid, the precursor for these indolenines, was developed.

Key words: indolenines, 2-acetylcyclohexanone, oxo acids, heterocyclization.

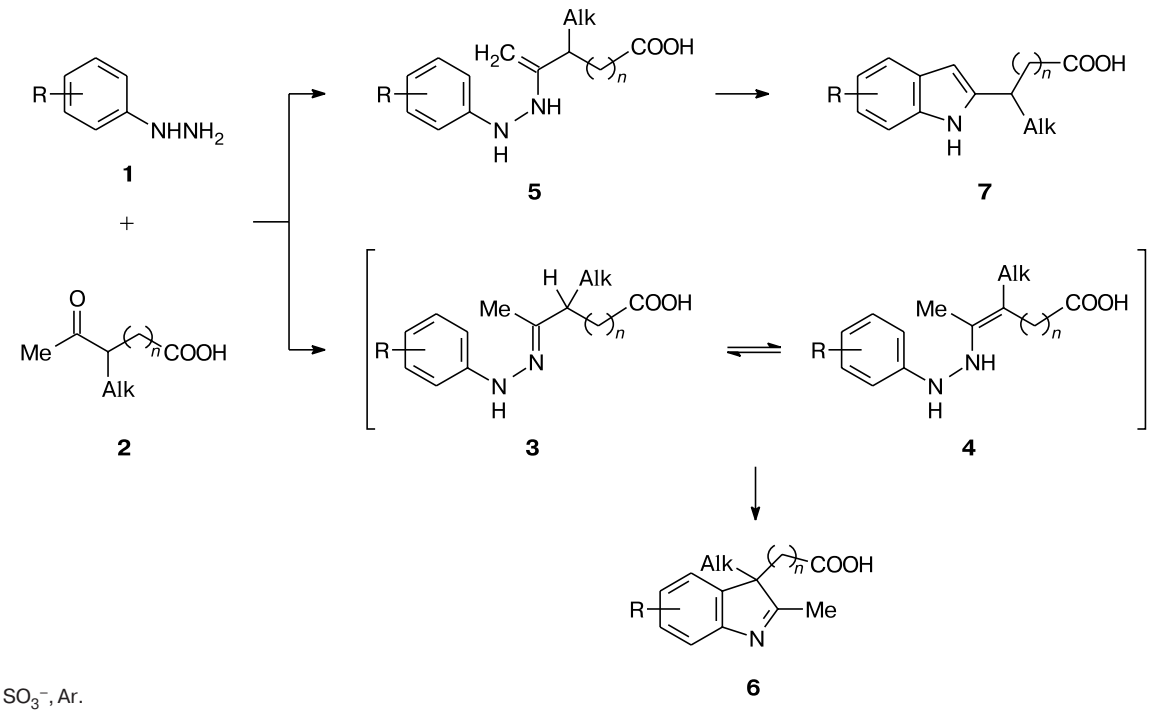
2-Methylindolenines are important intermediates in the synthesis of indocyanine dyes used as fluorescent labels for biological analysis.[1—5] The properties of fluorescent labels largely depend on the type and location of various substituents. The position of the reactive carboxy group in the indolenine fragment affects the spatial orientation of the label relative to a labeled biomolecule (protein or oligonucleotide).

For the synthesis of dyes with a carboxyl function, carboxyl-containing indoleninium bases are used. They can be prepared in several ways. *N*-Alkylation of indolenines with ω-bromo carboxylic acids gives the corresponding 1-(ω-carboxyalkyl)indoleninium salts.[6] Compounds with the carboxy group in position 5 are synthesized by the Fischer method from carboxyl-containing phenylhydrazines and isopropyl methyl ketone.[7]

3-Carboxyalkylindolenines are of particular interest. A hydrocarbon chain between the carboxyl function and the heterocycle diminishes their reciprocal influence.[8,9] The present study was devoted to the synthesis of indolenines from 6-methyl-7-oxooctanoic acid.

The Fischer synthesis is the most accessible route to indolenines (Scheme 1). Condensation of various phenylhydrazines **1** with oxo acids **2** in acidic media involves

Scheme 1



R = Alk, SO_3^-, Ar.

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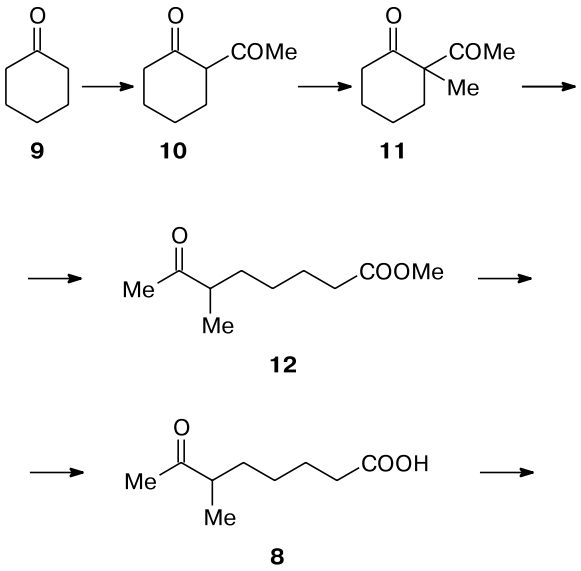
two sequential reactions: the formation of hydrazones **3** or **5** and their cyclization. Arylhydrazones of ketones with an α-branched alkyl chain are known to undergo cyclization only at the tertiary C atom to give 2-methylindolenines **6**.[10] This cyclization pathway is preferred because ene hydrazine **4** is more stable (contains more substituents at the double bond) than ene hydrazine **5**.

The heterocyclization pathway is strongly influenced by the acidity of the medium. For instance, $ZnCl_2$, AcOH, the bisulfate ion, and aqueous H_3PO_4 always yield cyclization products in the more branched chain of the ketone (compounds **6**). Polyphosphoric acid and conc. H_2SO_4 and HCl give mixtures of products, the yields of compounds **7** increasing with the acidity of the medium.

For the synthesis of 3-carboxyalkylindolenines, an appropriate oxo carboxylic acid (of the type **2**) with the terminal carboxy group is required. Such acids can be prepared in several ways. 7-Methyl-8-oxononanoic acid has been synthesized from ethyl 2-methylacetoacetate and ethyl 6-bromohexanoate.[11] Pritzkow *et al*. have proposed to obtain methyl 6-methyl-7-oxooctanoate from *N*-nitrosocaprolactam.[12] The multistep scheme including the Claisen retrocondensation of various 2-acetylcycloalkanones affords oxo acids **2**.[13] It should be noted that the retrocondensation outcome is sensitive to the ring size and the reaction conditions. We developed a convenient method for the synthesis of oxo acid **8** from cyclohexanone (**9**) (Scheme 2).

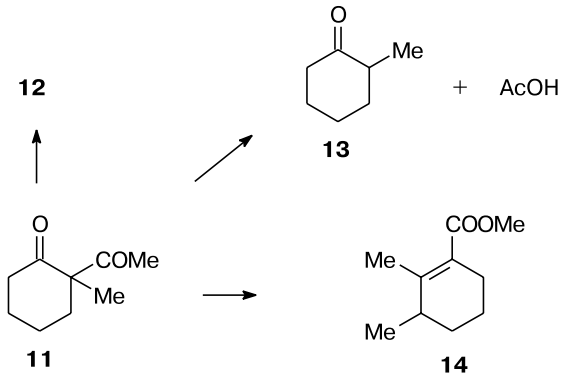
Scheme 2



2-Acetyl-2-alkylcyclohexanones can be prepared in several ways.[13–15] The degree of methylation at position 2 of the ring depends on the alkylating agent and the reaction conditions. We methylated 2-acetylcyclohexanone[16,17] (**10**) with MeI in the presence of aqueous NaOH [15] to give 2-acetyl-2-methylcyclohexanone (**11**) in 75% yield. This method is very simple. The presence of unreacted ketone **10** in the product was controlled by a color reaction with $FeCl_3$ in EtOH (a qualitative reaction for enols).

We found that diketone **11** reacts with bases to give three major products rather than two ones reported earlier[13,18,19] (Scheme 3).

Scheme 3



The reaction in aqueous 10% NaOH mainly resulted in deacetylation of compound **11**: the yield of 2-methylcyclohexanone (**13**) was 60%, while the yield of acid **8** was less than 10%. The reaction in the presence of MeONa afforded compounds **12**, **13**, and **14** in 35, 15, and 30% yields, respectively. The best results were obtained with MeOLi. The total yield of by-products **13** and **14** was lowered to 30%, and oxo ester **12** was isolated by vacuum distillation in 52% yield.

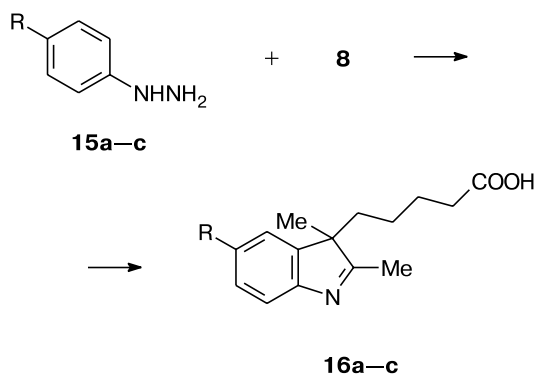
We found optimum conditions for the acid hydrolysis of ester **12**. The highest yield (75%) of oxo acid **8** was achieved with 30% HCl.[20]

Further reactions of oxo acid **8** with phenylhydrazines **15a—c** were carried out in boiling AcOH in one step, without isolating intermediate phenylhydrazones **3** (see Refs 6—8 and Schemes 1, 4).

Additional recrystallization from Et_2O—hexane (**16a**) or AcOEt—hexane (**16c**) gave the indolenines in 78 and 84% yields, respectively. Water-soluble indolenine **16b** was converted into its potassium salt under the action of KOH in MeOH (see Ref. 6) and isolated in 83% yield.

To sum up, we obtained novel carboxyl-containing indolenines **16a—c**, which can serve as intermediates for the synthesis of cyanine dyes. The presence of a carboxyalkyl group in position 3 and various substituents in position 5 of indolenine provides great scope for the preparation of new efficient fluorescent labels.

Scheme 4



R = Me (**15a**, **16a**), SO_3H (**15b**), SO_3K (**16b**), H (**15c**, **16c**)

Experimental

^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-400 pulse Fourier spectrometer (Germany) in $CDCl_3$ (with Me_4Si as the external standard) and D_2O. MALDI-TOF mass spectra were recorded on a Kompact MALDI 4 instrument (Kratos Analytical, US) with 2,5-dihydroxybenzoic acid as a matrix for low-molecular-weight organic compounds. Mass spectra of low-molecular-weight compounds were recorded on a Kratos MS-30 instrument (230 °C, 70 eV). Melting points were determined on a Kofler Boetius hot stage (Germany). UV spectra were recorded on a Jasco V-550 spectrophotometer (Japan).

4-Tolylhydrazine hydrochloride,[21] 4-hydrazinobenzenesulfonic acid,[22] and 2-acetylcyclohexanone[16,17] were prepared according to known procedures.

2-Acetyl-2-methylcyclohexanone (11). A three-neck round-bottom flask fitted with a reflux condenser, a stirrer, and a thermometer was charged with MeOH (83 mL), 2-acetylcyclohexanone (**10**) (29.0 g, 26.9 mL, 0.21 mol), and MeI (44.0 g, 19.3 mL, 0.31 mol). A solution of NaOH (8.3 g, 0.21 mol) in water (41.4 mL) was added dropwise at 0 °C to the stirred reaction mixture. The mixture was stirred at ~20 °C for 20 h and then refluxed for 2 h. On cooling, water (85 mL) was added and the product was extracted with chloroform (2×85 mL). The organic extracts were combined, washed with 2 *M* NaOH (200 mL) and brine (100 mL), and dried with $MgSO_4$. The solvent was removed and the residue was fractionally distilled under nitrogen *in vacuo*. The yield of compound **11** was 23.8 g (75%), b.p. 127—128 °C (32 Torr) (*cf.* Ref. 15: b.p. 74—76 °C (3 Torr)).

^1H NMR ($CDCl_3$), δ: 1.23 (s, 3 H, C(2)CH_3); 1.41—1.48 (m, 1 H, H(4)); 1.61—1.69 (m, 3 H, H(4), $H_2C(5)$); 1.93—1.99 (m, 1 H, H(3)); 2.09 (s, 3 H, $COCH_3$); 2.25—2.34 (m, 1 H, H(3)); 2.42—2.48 (m, 2 H, $H_2C(6)$). ^{13}C NMR ($CDCl_3$), δ: 20.76 (2-CH_3); 22.31 (C(4)); 25.61 (CO<u>C</u>H_3); 27.26 (C(5)); 36.80 (C(3)); 41.15 (C(6)); 63.76 (C(2)); 207.56 (<u>C</u>OCH_3); 210.26 (C(1)).

Methyl 6-methyl-7-oxooctanoate (12). A mixture of anhydrous MeOH (52.4 mL), metallic Li (0.5 g, 0.071 mol), and 2-acetyl-2-methylcyclohexanone (**11**) (10.0 g, 0.065 mol) was refluxed with stirring for 1 h and then kept at room temperature for 12 h. Then the reaction mixture was acidified with conc. H_2SO_4 to pH 4—5. Water (55 mL) was added to the resulting suspension and the product was extracted with ether until the aqueous phase became colorless. The organic extracts were combined, washed with brine (100 mL), and dried with $MgSO_4$. The solvent was removed and the residue was fractionally distilled *in vacuo* to give three products **12**, **13**, and **14**.

2-Methylcyclohexanone (13). The yield was 0.8 g (11%), b.p. 51—52 °C (22 Torr) (*cf.* Ref. 13: b.p. 165—166 °C). ^1H NMR ($CDCl_3$), δ: 1.02 (d, 3 H, C(2)CH_3, J = 6.5 Hz); 1.37, 1.81 (m, 2 H, $H_2C(4)$); 1.65 (m, 2 H, $H_2C(3)$); 2.05 (m, 2 H, $H_2C(5)$); 2.36 (m, 2 H, H_2C (6)).

Methyl 2,3-dimethylcyclohex-1-enecarboxylate (14). The yield was 2.1 g (19%), b.p. 125—130 °C (20 Torr). MS (EI, 70 eV), m/z (I_{rel} (%)): 168.0 [M^+] (100%). ^1H NMR ($CDCl_3$), δ: 0.97 (d, 3 H, C(3)CH_3, J = 7 Hz); 1.31 (s, 3 H, C(2)CH_3); 1.49—1.78 (m, 4 H, $H_2C(4)$, $H_2C(5)$); 1.87 (m, 3 H, H(3), $H_2C(6)$); 3.69 (s, 3 H, COOC<u>H</u>$_3$). ^{13}C NMR ($CDCl_3$), δ: 14.34 (3-CH_3); 19.57 (2-CH_3); 21.23 (C(5)); 26.78 (C(6)); 36.06 (C(4)); 46.52 (C(3)); 49.42 (COO<u>C</u>H_3); 86.73 (C(1)); 121.20 (C(2)); 178.78 (<u>C</u>OOCH_3).

Methyl 6-methyl-7-oxooctanoate (12). The yield was 6.2 g (52%), b.p. 139—143 °C (20 Torr) (*cf.* Ref. 18: b.p. 120—124 °C (12 Torr)). ^1H NMR ($CDCl_3$), δ: 1.06 (d, 3 H, C(6)CH_3, J = 7 Hz); 1.23—1.35 (m, 3 H, $H_2C(5)$, H(4)); 1.56—1.69 (m, 3 H, $H_2C(3)$, H(4)); 2.11 (s, 3 H, $H_3C(8)$); 2.28 (t, 2 H, $H_2C(2)$, J = 7.5 Hz); 2.49 (m, 1 H, H(6)); 3.63 (s, 3 H, COOC<u>H</u>$_3$).

6-Methyl-7-oxooctanoic acid (8). A mixture of ~12% HCl (37 mL) and methyl 6-methyl-7-oxooctanoate (**12**) (9.1 g, 0.049 mol) was refluxed with stirring for 6 h. The product was extracted with ether (50 mL), the solvent was removed, and the residue was fractionally distilled *in vacuo*. The yield of acid **8** was 5.9 g (75%), b.p. 122 °C (0.1—0.01 Torr) (*cf.* Ref. 13: b.p. 128—129 °C (0.7 Torr)). ^1H NMR ($CDCl_3$), δ: 1.06 (d, 3 H, C(6)CH_3, J = 7 Hz); 1.25—1.39 (m, 3 H, $H_2C(5)$, H(4)); 1.58—1.69 (m, 3 H, $H_2C(3)$, H(4)); 2.12 (s, 3 H, $H_3C(8)$); 2.33 (t, 2 H, $H_2C(2)$, J = 7.5 Hz); 2.50 (m, 1 H, H(6)). ^{13}C NMR ($CDCl_3$), δ: 16.29 (6-CH_3); 19.57 (2-CH_3); 24.73 (C(3)); 26.72 (C(4)); 28.08 (C(8)); 32.45 (C(5)); 33.92 (C(2)); 47.02 (C(6)); 179.55 (COOH); 213.09 (C(7)).

3-(4-Carboxybutyl)-2,3,5-trimethylindolenine (16a). A mixture of 4-tolylhydrazine hydrochloride (**15a**) (825 mg, 5.2 mmol), glacial AcOH (8 mL), 6-methyl-7-oxooctanoic acid (**8**) (913 mg, 5.3 mmol), and anhydrous AcOK (980 mg, 10 mmol) was refluxed with stirring for 6 h. The solvent was removed and the residue was dissolved in chloroform (10 mL). The resulting solution was washed with water (20 mL) and brine (20 mL) and dried with $MgSO_4$. The solvent was removed and the residue was recrystallized from Et_2O—hexane (1 : 10). The yield of indolenine **16a** was 1.13 g (84%), dark orange crystals. UV (MeOH), λ_{max}/nm: 254; m.p. 138—140 °C. Found: m/z 260.7 $[M]^+$. $C_{16}H_{21}NO_2$. Calculated: M = 259.34. ^1H NMR ($CDCl_3$), δ: 0.60—0.83 (m, 2 H, CH_2CH_2C<u>H</u>$_2CH_2COOH$); 1.25 (s, 3 H, $H_3C(3)$); 1.46 (m, 2 H, C<u>H</u>$_2CH_2CH_2CH_2COOH$); 1.72—1.88 (m, 2 H, CH_2C<u>H</u>$_2CH_2CH_2COOH$); 2.09—2.18 (m, 2 H, $CH_2CH_2CH_2C$<u>H</u>$_2COOH$); 2.28 (s, 3 H, $H_3C(2)$); 2.37 (s, 3 H, $H_3C(5)$); 7.01—7.40 (m, 3 H, Ar); 10.11 (s, 1 H, $CH_2CH_2CH_2CH_2COO$<u>H</u>).

Potassium 3-(4-carboxybutyl)-2,3-dimethylindolenine-5-sulfonate (16b). A mixture of 4-hydrazinobenzenesulfonic acid (**15b**) (1.8 g, 9.7 mmol), 6-methyl-7-oxooctanoic acid (**8**) (2.0 g, 11.6 mmol), and glacial AcOH (15 mL) was refluxed with stirring for 8 h. Then another portion of AcOH (15 mL) was added and the mixture was treated with activated charcoal. The solvent was removed and the residue was dissolved in MeOH (10 mL). A solution of KOH (600 mg, 10.6 mmol) in MeOH (7 mL), Pr^iOH

(15 mL), and Et_2O (2 mL) was added dropwise. The precipitate that formed was filtered off and dried in a vacuum desiccator over P_2O_5. The yield of potassium salt **16b** was 3.5 g (83%), light beige crystals. UV (MeOH), λ_{max}/nm: 258; m.p. 198—200 °C. Found: *m/z* 327.2 $[M - K]^+$. $C_{15}H_{19}NO_5S$. Calculated: M = 325.38. ^{1}H NMR (D_2O), δ: 0.45—0.70 (m, 2 H, $CH_2CH_2C\underline{H}_2CH_2COOH$); 1.31 (s, 3 H, $H_3C(3)$); 1.36 (m, 2 H, $C\underline{H}_2CH_2CH_2CH_2COOH$); 1.88—2.14 (m, 4 H, $CH_2C\underline{H}_2CH_2C\underline{H}_2COOH$); 7.53 (d, 1 H, Ar, *J* = 9 Hz); 7.80 (m, 2 H, Ar).

3-(4-Carboxybutyl)-2,3-dimethylindolenine (16c). A mixture of 6-methyl-7-oxooctanoic acid (**8**) (723 mg, 4.2 mmol), phenylhydrazine (**15c**) (454 mg, 414 μL, 4.2 mmol), and glacial AcOH (4.2 mL) was heated in an inert atmosphere at 118 °C for 3.5 h. The solvent was removed and the oily residue was dissolved in chloroform (10 mL). The resulting solution was washed with water (20 mL) and brine (20 mL), dried with $MgSO_4$, and concentrated. The residue was recrystallized from AcOEt—hexane (0.6 : 2). The yield of indolenine **16c** was 0.8 g (78%), yellowish orange crystals. UV (MeOH), λ_{max}/nm: 256; m.p. 112—113 °C. Found: *m/z* 246.3 $[M]^+$. $C_{15}H_{19}NO_2$. Calculated: M = 245.32. ^{1}H NMR ($CDCl_3$), δ: 0.56—0.81 (m, 2 H, $CH_2CH_2C\underline{H}_2CH_2COOH$); 1.28 (s, 3 H, $H_3C(3)$); 1.43—1.51 (m, 2 H, $C\underline{H}_2CH_2CH_2CH_2COOH$); 1.76—1.92 (m, 2 H, $CH_2C\underline{H}_2CH_2CH_2COOH$); 2.13—2.21 (m, 2 H, $CH_2CH_2CH_2C\underline{H}_2COOH$); 2.24 (s, 3 H, $H_3C(2)$); 7.18—7.54 (m, 4 H, Ar).

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