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ALKALOIDS OF *MELODINUS GUILLAUMINII**

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Key Word Index—*Melodinus guillauminii*, Apocynaceae, indole alkaloids, stem bark, aerial parts

Abstract—Fourteen alkaloids were isolated from the stem bark and aerial parts of *Melodinus guillauminii* They were 11-hydroxytabersonine, venalstonine, venalstonidine, 14,15-seco-3-oxokopsinal, 3-oxovenalstonine, 11-methoxy-Δ^{14}-vincamenine, 3-oxo-hydroxykopsinine, 11-methoxy-Δ^{14}-vincanol, kopsinine, 15-α-hydroxykopsinine, 11-methoxy-tabersonine, pleiocarpamine, 19-β-hydroxyvenalstonine and guillauminine

INTRODUCTION

As part of a chemotaxonomic study of New Caledonian plants, we describe in the present report the alkaloids isolated from the stem bark and aerial parts of *Melodinus guillauminii* Boiteau collected and identified by two of us (T S and J P) This species is classified in the *Bicorona* section according to Boiteau *et al* [1]

RESULTS AND DISCUSSION

Extractions were performed in the usual manner and the yield of alkaloid mixture was 8 2% (g/kg) from the stem bark and 1 8% from the aerial parts The alkaloids were separated by column chromatography and preparative TLC Eleven alkaloids were isolated from the stem bark They were, by order of increasing polarity on TLC, 11-hydroxytabersonine (**1**) (8% of alkaloid mixture), venalstonine (**2**) (5%), venalstonidine (**3**) (5%), 14,15-seco-3-oxokopsinal (**4**) (0 5%), 3-oxovenalstonine (**5**) (5%), 11-methoxy-Δ^{14}-vincamenine (**6**) (1%), 3-oxo-hydroxykopsinine (**7**) (1%), 11-methoxy-Δ^{14}-vincanol (**8**) (1%), guillauminine (**9**) (0 5%), kopsinine (**10**) (15%) and 15-α-hydroxykopsinine (**11**) (15%) Known alkaloids **1**, **2** and **10** were identified by direct comparison with authentic samples Alkaloids **3**, **5** and **11** were identified by comparison of their spectral and physical properties with literature data [2, 3] The structures of the novel alkaloids **4**, **6**, **8** were established as follows

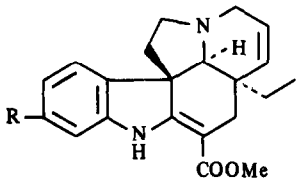
The mass spectrum of **4** showed a $[M]^+$ at *m/z* 368 ($C_{21}H_{24}O_4N_2$) and fragment-ions (Scheme 1) at *m/z* 227 (**a**), 214 (**b**), 195 (**c**), 168 (**d**) and 154 (**e**) These fragments were also found in the mass spectra of 3-oxovenalstonine (**5**) [2], 3-oxotabersonine [4] and 3-oxovincadifformine [5] The UV spectrum of **4**, the absence an ethyl side-chain pattern in the ^{1}H NMR spectrum and the presence in the mass spectrum of a typical kopsine fragmentation [6] ($[M-28]^+$) ruled out a tabersonine-like structure The IR and ^{1}H NMR spectra of **4** confirmed the presence of four oxygen atoms included in a carbomethoxy unit ($\nu_{C=O}$ 1735 cm^{-1}, 3-proton singlet at δ 3 75), in a *N*-acetyl group ($\nu_{C=O}$ 1650 cm^{-1}, 3-proton singlet at δ 2 15) and in an aldehyde ($\nu_{C=O}$ 1720 cm^{-1}, 1-proton singlet at δ 9 6) The isomeric structure **12** was ruled out by the chemical shift of the aldehyde proton as well as by sodium borohydride reduction of **4** to **13** in which the *N*-acetyl group was unaffected

11-Methoxy-Δ^{14}-vincanol (**8**) showed a $[M]^+$ at *m/z* 324 and its mass spectrum was similar to those of 16-epi-Δ^{14}-vincanol (**14**) [7, 8] and Δ^{14}-vincanol (**15**) [9] The main fragments of **8** (*m/z* 306, 295, 277, 256, 238) were shifted by 30 amu from the corresponding fragments of **15** According to the ^{1}H NMR spectrum, a methoxy (3-proton singlet at δ 3 85) was located either on C-10 or C-11 to account for an AMX spectrum with J_{AM} = 8 Hz (*ortho*), J_{MX} = 1 5 Hz (*meta*), J_{AX} = O (*para*) Besides a broad singlet for two olefinic protons (H-14 and H-15), the ^{1}H NMR spectrum showed the H-16 signal as a doublet of doublets ($J_{H\text{-}16,H\text{-}17a}$ = 10 5 Hz, $J_{H\text{-}16,H\text{-}17e}$ = 4 5 Hz) indicating a quasi-axial orientation of this proton as in eburnamine [10] Jones oxidation of **8** yielded **16**, in which H-12 underwent the deshielding anisotropic effect of the carbonyl (δ 7 90) The observation of a *meta*-coupling on this proton fixed the methoxy-substituent on C-11 in **16** and hence in **8**

11-Methoxy-Δ^{14}-vincamenine was suggested for alkaloid **6** because of its spectral analogies with 11-methoxy-Δ^{14}-vincanol (**8**), with eburnamenine [11] and with Δ^{14}-vincamenine (**17**) [9] The mass spectrum of **6** showed ions at *m/z* 277 and 238, also present in the spectrum of **8**, but the $[M]^+$ (*m/z* 306) was 18 amu lower The ^{1}H NMR spectrum showed a methoxy group and a set of four olefinic protons giving the same pattern as the olefinic protons of Δ^{14}-vincamenine (**17**) H-16 (*d*, *J* = 9 Hz, δ6 9), H-14 and H-15 (*m*, δ5 4), H-17 (*d*, *J* = 9 Hz, δ4 9)

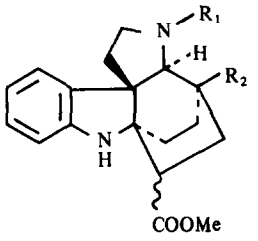
Structures **6** and **8** were also supported by the following chemical correlations Δ^{14}-16-Epivincine (**18**) was reacted with *t*-BuOK in benzene to give Δ^{14}-vincinone (**16**) which was reduced ($LiAlH_4$) to a mixture, the major compound of which was identical to **8** (TLC, $[\alpha]_D$, UV, IR, MS,

*Part 85 in the series "Plants from New Caledonia" For Part 84 see Massiot, G, Guillaume, D, Vercauteren, J and Le Men-Olivier, L (1983) *C R Acad Sci Ser* II **296**, 977



1 R = OH

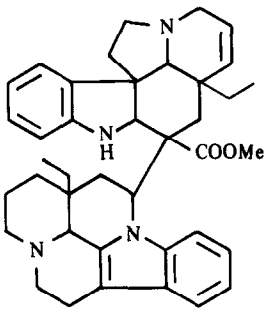
20 R = OMe



4 R_1 = COMe, R_2 = CHO

12 R_1 = CHO, R_2 = COMe

13 R_1 = COMe, R_2 = CH_2OH



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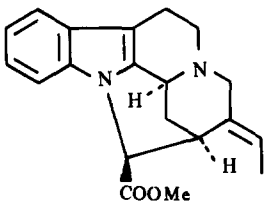

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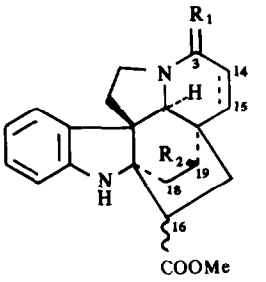


2 $R_1 = H_2, R_2 = H, \Delta^{14}$, 16 β H

3 $R_1 = H_2, R_2 = H$, 14 α, 15 α epoxy, 16 β H

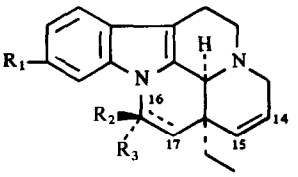
5 $R_1 = O, R_2 = H, \Delta^{14}$, 16 β H

7 $R_1 = O, R_2 = H$, + OH indetermined

10 $R_1 = H_2, R_2 = H$, 16 β H

11 $R_1 = H_2, R_2 = H$, 15 α OH, 16 β H

21 $R_1 = H_2$ $R_2 = OH, \Delta^{14}$, 16 β H



6 R_1 = OMe, Δ^{16}

8 R_1 = OMe, R_2 = H, R_3 = OH

14 R_1 = H, R_2 = OH, R_3 = H

15 R_1 = H, R_2 = H, R_3 = OH

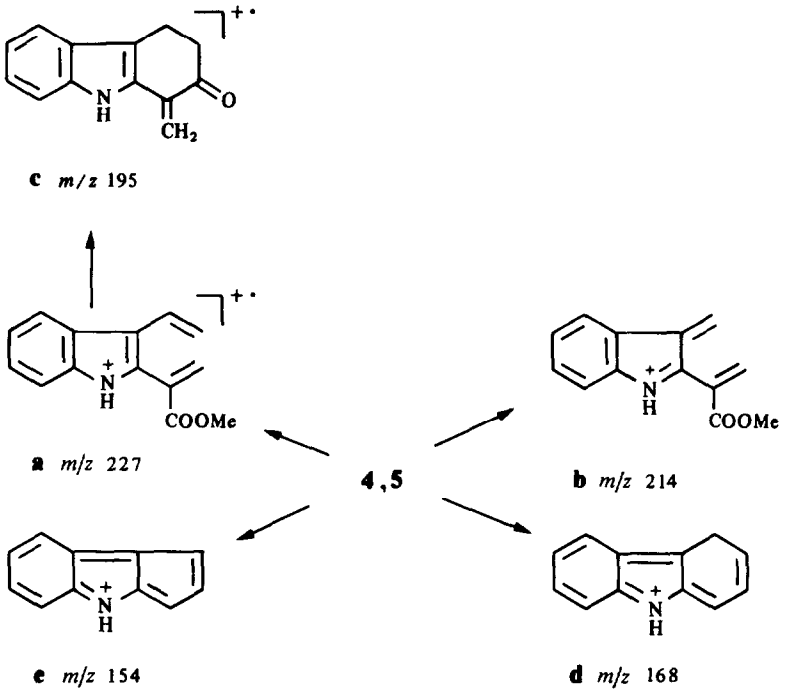
16 R_1 = OMe, R_2, R_3 = O

17 R_1 = H, Δ^{16}

18 R_1 = OMe, R_2 = COOMe, R_3 = OH

NMR) Dehydration of **8** yielded 11-methoxy-Δ^{14}-vincamenine identical (TLC, UV, IR, MS, NMR) with alkaloid **6**

The novel alkaloids **7** and **9** were only isolated in minute quantities and the structure or elements of structure we propose for them are provisional Examination of the spectra of **7**, and especially of its mass spectrum (ions **a**, **b**, **c**, **d**, **e**) suggested a 3-oxovenalstonine skeleton bearing an additional 18 amu substituent ($[M]^+$ at m/z 368) The lack of any olefinic proton (NMR) and the presence of a hydroxyl group (IR, NMR) suggested 3-oxo-hydroxykopsinine (**7**) for its structure The absence in the mass spectrum of a $[M-28]^+$ fragment indicated that the hydroxyl group might be present on C-18 or C-19



Scheme 1 Mass spectral fragmentation of 14,15-seco-3-oxokopsinal (4)

Guillauminine (9) was a bisindole alkaloid ($[M]^+$ *m/z* 616, $C_{40}H_{48}N_4O_2$; HRMS) whose mass spectrum was reminiscent of that of isomeric paucivenine (19) [12] The IR spectrum of 9 showed no carbonyl absorption The complexity of the UV spectrum (maxima at 214, 236, 255 (sh), 280 (sh) and 303) agreed with the superimposition of 11-methoxyindole and of dihydroindole chromophores The ^{1}H NMR spectrum (400 MHz) showed five aromatic protons Three of them formed an AMX system compatible with a substitution on C-10 or C-11, the two remaining aromatic protons were singlets suggesting a double substitution on the aromatic nucleus at C-10′ and C-11′ The three aromatic substituents were a methoxy (3-proton singlet at δ 3 53) and a methylene (2-proton singlet at δ 3 86) linking the two aromatic parts of the dimer Two ethyl chains and four olefinic protons were also detected together with a doublet of doublets at δ 5 2 which was reminiscent of the H-16 of 11-methoxy-Δ^{14}-vincanol (8) These data suggested that guillauminine contained a moiety similar to 8 The extra methylene, which links the two moieties of the dimer, is not readily accounted for by the currently accepted biogenesis of the indole alkaloids However, a precedent for the presence of such an extra carbon atom is found in vindolicine [13] It is not unreasonable to think that the addition of this carbon atom occurs during the extraction procedure

The alkaloid content of the aerial parts was almost identical to that of the stem bark Twelve alkaloids were isolated, which were, by order of increasing polarity, 11-methoxytabersonine (20) (0 5% of alkaloid mixture), 11-hydroxytabersonine (1) (10%), venalstonine (2) (0 5%), venalstonidine (3) (0 5%), 3-oxovenalstonine (5) (0 5%), 11-methoxy-Δ^{14}-vincamenine (6) (1%), 3-oxo-hydroxykopsinine (7) (1%), 19-β-hydroxyvenalstonine (21) (5%), 11-methoxy-Δ^{14}-vincanol (8) (3%), guillauminine (9) (3%), pleiocarpamine (22) (1%) and kopsinine (10) (1 5%) Known alkaloids 20 and 22 were identified by direct comparison with authentic samples

The new 19-β-hydroxyvenalstonine (21) was isolated at the same time by S Baassou from *M reticulatus* and its structure was independently established Full details for the structure elucidation of 21 are found in ref [9], physical and spectral properties of our material are reported in the Experimental

Conclusion

M guillauminii is a novel species introduced by P Boiteau As in all the plants of this genus studied so far, the alkaloids belong to type β of Le Men-Taylor classification The two subtypes aspidospermane-kopsane and vincane are also present in *M celastroides*, which belongs to the same *Bicorona* section according to Pichon It is worth noting, however, that these two species bear no botanical ressemblance

EXPERIMENTAL

General Optical rotations were determined in $CHCl_3$ NMR spectra were measured in $CDCl_3$ solns at 60 MHz, chemical shifts are given in δ with TMS as int standard Chromatographic columns were packed with silica gel (Merck H 60) TLC plates were sprayed (CR) with a soln of $[Ce(IV)(NH_4)_2]SO_4$. Plant material (Sevenet 470 and Sevenet-Pusset 1714) was collected in the Dome of Tiebaghi in 1973

Extraction of stem bark Bark (800 g), alkalized with NH_4OH, was lixiviated with 20 l EtOAc The lixiviate was extracted with 2% H_2SO_4 and the aq phase made alkaline with NH_4OH and extracted with $CHCl_3$ The $CHCl_3$ layer was dried (Na_2SO_4) and evapd *in vacuo* to give 6 6 g of crude alkaloid

mixture (8 2 g/kg) The mixture was placed on a silica gel column (200 g) packed with $CHCl_3$ (6 5 1), $CHCl_3$–MeOH (99 1) (3 1), $CHCl_3$–MeOH (49 1) (1 2 1), $CHCl_3$–MeOH (19 1) (1 8 1) and $CHCl_3$–MeOH (9 1) (2 1) Fractions 9–11 (234 mg) contained 11-methoxy-Δ^{14}-vincamenine (**6**) and 3-oxovenalstonidine (**5**), fractions 12–18 (168 mg) contained venalstonidine (**3**), 14,15-seco-3-oxokopsinal (**4**) and **5**, fractions 19–21 (245 mg) contained venalstonine (**2**), **3**, **4**, **5** and 3-oxo-hydroxykopsinine (**7**), fractions 22–26 (368 mg) contained 11-hydroxytabersonine (**1**), fractions 27–29 (613 mg) contained 11-methoxy-Δ^{14}-vincanol (**8**), guillauminine (**9**) and kopsinine (**10**), fractions 52–55 (1030 mg) contained 15-α-hydroxykopsinine (**11**) The alkaloids were finally purified by TLC (EtOAc–*iso*-PrOH, 99 1 or 19 1)

Extraction of aerial parts Aerial parts were finely ground and extracted as previously described 9 g of crude alkaloid mixture was obtained (1 8 g/kg) which was purified on a silica gel column (270 g) packed with $CHCl_3$ (fractions of 400 ml) Each fraction showed a very complex mixture on TLC and the alkaloids were purified by prep TLC 11-Methoxytabersonine (**20**), venalstonine (**2**), venalstonidine (**3**), 3-oxovenalstonine (**5**), 11-methoxy-Δ^{14}-vincamenine (**6**) and 3-oxo-hydroxykopsinine (**7**) were obtained in fraction 5 (1393 mg), **2**, **3**, **5** and 11-hydroxytabersonine (**1**) in fraction 6 (627 mg), guillauminine (**9**) in fraction 13 (110 mg) and in fractions 14 and 15 (507 mg) with 19-β-hydroxyvenalstonine (**21**) and 11-methoxy-Δ^{14}-vincanol (**8**) Fractions 16 and 17 (1121 mg) contained **8**, **9**, **21** and kopsinine (**10**) Fractions 23–25 contained pleiocarpamine (**22**)

14,15-*Seco*-3-*oxokopsinal* (**4**) (CR orange) $[\alpha]_D$ −41° (*c* 0 4), UV λ_{max}^{MeOH} nm 213, 245, 295, IR ($CHCl_3$) cm^{-1} 3400, 2880, 1735, 1720, 1650, 1620, MS *m/z* (rel int) 368 (45), 340 (20), 325, 309, 296, 255, 227 (100), 215, 214 (50), 195 (64), 183, 168, 156, 154, 130; ^1H NMR δ 9 6 (*s*, 1H), 7 2–6 4 (*m*, 4H), 4 4 (*br s*, 1H), 3 85 (*s*, 1H), 3 75 (*s*, 3H), 2 15 (*s*, 3H)

11-*Methoxy*-Δ^{14}-*vincamenine* (**6**) (CR yellow), UV λ_{max}^{MeOH} nm 210, 227, 267, 290 (sh), 300 (sh), IR ($CHCl_3$) cm^{-1} 1640, MS *m/z* (rel int) 306 (100), 277 (100), 247 (80), 238 (75), ^1H NMR δ 7 5–6 5 (*m*, 4H), 6 9 (*d*, 1H, *J* = 8 Hz), 5 4 (*m*, 2H), 4 9 (*d*, 1H, *J* = 8 Hz), 4 35 (*s*, 1H), 1 0 (*t*, 3H, *J* = 7 Hz)

3-*Oxo-hydroxyvenalstonine* (**7**) (CR orange), $[\alpha]_D$ −14 5° (*c* 0 15), UV λ_{max}^{MeOH} nm 245, 290, IR ($CHCl_3$) cm^{-1} 3300, 1735, 1640, MS *m/z* (rel int) 368 (30), 227 (100), 214 (50), 195 (60), 182, 168, 156, 154, ^1H NMR δ 7 6–6 6 (*m*, 4H), 4 25 (*m*, 2H), 3 8 (*s*, 1H), 3 75 (*s*, 3H), 3 5 (*s*, 1H)

11-*Methoxy*-Δ^{14}-*vincanol* (**8**) (CR yellow), $[\alpha]_D$ +111° (*c* 0 4), UV λ_{max}^{MeOH} nm 213, 233, 267 (sh), 276, 300, IR ($CHCl_3$) cm^{-1} 3360, 1620, MS *m/z* (rel int) 324 (50), 306 (33), 295 (50), 277 (100), 256 (20), 249 (25), 238 (25), 223, ^1H NMR δ 7 3 (*d*, 1H, *J* = 8 Hz), 7 26 (*d*, 1H, *J* = 1 5 Hz), superimposed on $CHCl_3$), 6 75 (*dd*, 1H, *J* = 8, 1 5 Hz), 5 45 (*br s*, 2H), 5 2 (*dd*, 1H, *J* = 10 5, 4 5 Hz), 3 9 (*s*, 1H), 3 85 (*s*, 3H), 0 95 (*t*, 3H, *J* = 7 Hz)

Guillauminine (**9**) (CR violet), $[\alpha]_D$ +186° (*c* 1), UV λ_{max}^{MeOH} nm 214, 236, 255 (sh), 280 (sh), 303, IR ($CHCl_3$) cm^{-1} 3400, 1620, MS *m/z* (rel int) 616 (88), 587, 548, 546, 416 (14), 387 (10), 336 (96), 323 (20), 308 (10), 307 (16), 306 (16), 280 (100), 279 (44), 277 (12), 265, 251, 238, 200, 173 (24), 134 (40), 121 (24), 107 (28) ^1H NMR (400 MHz) δ 7 32 (*d*, 1H, *J* = 8 Hz), 6 86 (*s*, 1H), 6 69 (*dd*, 1H, *J* = 8, 1 5 Hz), 6 35 (*s*, 1H), 6 1 (*d*, 1H, *J* = 1 5 Hz), 5 82 (*ddd*, 1H, *J* = 10, 5, 1 Hz), 5 62 (*br d*, 1H, *J* = 10 Hz), 5 57 (*ddd*, 1H, *J* = 10, 3, 2 Hz), 5 5 (*dd*, 1H, *J* = 10, 1 Hz), 5 2 (*br d*, 1H, *J* = 13 Hz), 4 15 (*s*, 1H), 3 86 (*s*, 2H), 3 53 (*s*, 3H), 0 97 (*t*, 3H, *J* = 7 Hz), 0 89 (*t*, 3H, *J* = 7 Hz)

19β-*Hydroxyvenalstonine* **21** (CR orange), $[\alpha]_D$ −39° (*c* 0 3), UV λ_{max}^{MeOH} nm 212, 245, 293, IR ($CHCl_3$) cm^{-1} 3450, 3360, 1730, 1610; MS *m/z* (rel int) 352 (100), 334, 321, 308 (40), 307 (20), 229 (20), 216 (94), 156 (32), 151 (63), 138, 123, 107 (80), ^1H NMR δ 7 2–6 55 (*m*, 4H), 5 8 (*br s*, 2H), 3 75 (*s*, 3H)

Reduction of **4** *to* **13** Alkaloid **4** (6 mg) was dissolved in 1 5 ml MeOH and KBH_4 (5 mg) was added The soln was stirred at room temp for 30 min The reaction mixture was then poured into H_2O and extracted with $CHCl_3$ After drying and evapn, 4 mg **13** was obtained which showed one spot on TLC UV λ_{max}^{MeOH} nm 213, 245, 293, IR ($CHCl_3$) cm^{-1} 3400, 1735, 1630, MS *m/z* (rel int) 370 (40), 352, 284, 254, 227 (100), 214, 212, 195, 168, 167, ^1H NMR δ 7 2–6 4 (*m*, 4H), 5 25 (*dd*, 1H, *J* = 12, 3 5 Hz exchangeable with D_2O), 4 2 (*br s*, 1H), 3 75 (*s*, 3H), 2 2 (*s*, 3H)

Oxidation of **8** *to* **16** Alkaloid **8** (7 mg) was dissolved in 1 ml Me_2CO and 1 drop of Jones reagent was added After 15 sec the mixture was neutralized with $NaHCO_3$ and extracted with Et_2O After drying, evapn and purification on TLC, **16** (2 mg) was obtained UV λ_{max}^{MeOH} nm 217, 250, 283, IR ($CHCl_3$) cm^{-1} 1700, MS *m/z* 322, 293, 265, 254, 217, ^1H NMR δ 7 90 (*d*, 1H, *J* = 1 5 Hz), 7 25 (*d*, 1H, *J* = 8 Hz), 6 85 (*dd*, 1H, *J* = 8, 1 5 Hz), 5 5 (*br s*, 2H), 4 1 (*br s*, 1H), 3 87 (*s*, 3H), 1 0 (*t*, 3H, *J* = 7 Hz)

Chemical correlations To a soln of Δ^{14}-16-epivincine (**18**) (100 mg) in C_6H_6 (3 ml) was added 100 mg *t*-BuOK The mixture was heated for 2 hr at 60° and concd *in vacuo* After addition of H_2O the mixture was extracted with CH_2Cl_2 After drying and evapn 42 mg **16** was obtained A portion of **16** (38 mg) was then reduced with $LiAlH_4$ in THF The reaction gave almost exclusively one compound identical to **8** (38 mg) It was dissolved in CH_2Cl_2 (3 ml) and 5 drops of TFA were added The soln was stirred for 20 min and extracted in the usual fashion The crude product (29 mg) was purified by TLC to give a compound (5 mg) identical to **6**

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