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DEHYDROISOSTRYCHNOBILINE, MATOPENSINE AND OTHER ALKALOIDS FROM *STRYCHNOS KASENGAENSIS*

PHILIPPE THEPENIER, MARIE-JOSE JACQUIER, GEORGES MASSIOT, LOUISETTE LE MEN-OLIVIER and CLEMENT DELAUDE*

Faculté de Pharmacie (E R A au C N R S N° 319) 51 rue Cognacq-Jay, 51096 Reims Cédex, France, *Université de Liège, Sart-Tilman, 4000, Liège, Belgium

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Key Word Index—*Strychnos kasengaensis*, Loganiaceae, indole alkaloids, ^{13}C NMR

Abstract—Eighteen alkaloids have been identified in the root bark, stem bark and leaves of *Strychnos kasengaensis* from Zaire, They are isoretulinal, retuline, desacetyl isoretuline, desacetyl retuline, dehydroisostrychnobiline, matopensine and its mono-*N*-oxide, nordihydrotoxiferine, isoretuline, N(1)-desacetyl 18-acetoxyisoretuline, Wieland-Gumlich aldehyde and diol, desacetyl isoretulinal, *O*-acetyl retuline, 16*R*-isositsirikine, *O*-acetyl isoretuline, 11-methoxy retuline and 11-methoxy isoretuline

INTRODUCTION

Strychnos kasengaensis De Wild is a liane from Eastern and central Africa [1], it belongs to the Lanigerae section of the genus *Strychnos* (Loganiaceae) As part of our studies on African *Strychnos* [2] we herein report our results on the alkaloid content of its stem bark, root bark and leaves

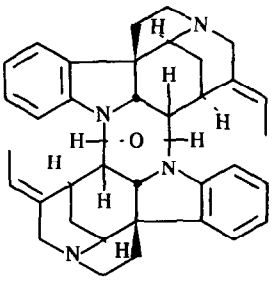
All extractions were conducted in the usual fashion [2], alkaloid mixtures (AM) were obtained with the following yields 13 8 g/kg (root bark), 6 0 g/kg (stem bark) and 1 3 g/kg (leaves) The root and stem bark AM were separated by medium pressure liquid chromatography, analytical samples were obtained by prep TLC of selected fractions The alkaloid content of the leaves was investigated by TLC The complexity of some of the fractions has precluded an accurate determination of the percentages of alkaloids and the figures given in brackets are approximate

RESULTS AND DISCUSSION

The major alkaloids isolated from the root bark are isoretulinal **1** (8% of AM) and retuline **2** (30% of AM) Isoretulinal **1** is one of the major alkaloids of *S variabilis* [3, 4] and it was identified by direct comparison, ^{13}C NMR, which was not available to us at the time of the first isolation is presented in the Experimental part The related desacetyl isoretuline **3** (2% of AM) and desacetyl retuline **4** (1% of AM) are also present Purification of the least polar fractions yields two minor bisalkaloids matopensine **5** (4% of AM) and dehydroisostrychnobiline **6** (5% of AM)

Matopensine **5** is a dimeric alkaloid, which is also present in *S matopensis* S Moore Its composition was determined as $C_{38}H_{42}N_4O$ by high resolution mass spectrometry (570 3366) The structure of **5**, a symmetrical dimer, was established by examination of its 400 MHz ^1H NMR spectrum, it has been the subject of a preliminary communication [5]

The molecular weight of the second nonpolar dimeric alkaloid **6** was determined as 612 by mass spectrometry, the $[M]^+$ analysed for $C_{40}H_{44}N_4O_2$ (meas 612 3442, calc 612 3462) The UV spectrum was the superimposition of several chromophores and showed maxima at 218, 250, 280 and 290 nm The IR spectrum showed no bands corresponding to OH or NH absorptions, a strong double band at 1650–1660 cm^{-1} seemed to indicate the



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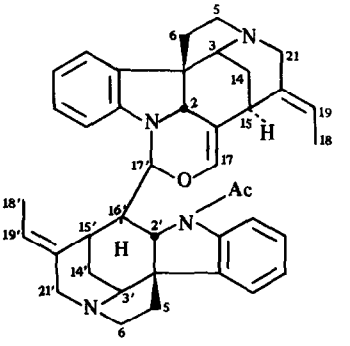
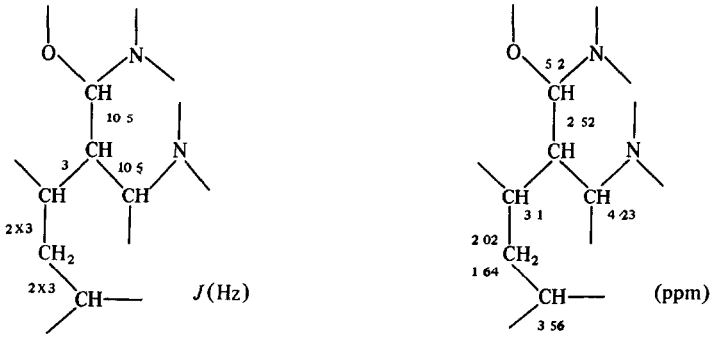


6

presence of an *N*-acyl function This was further confirmed by the 400 MHz ^{1}H NMR (Table 1), which showed a three-proton singlet at δ2 05 (Ac) and a one-proton doublet at 7 82 (H-12′), these features correspond to an N(1) acetyl group Two ethylidene side chains were also observed and one of them is unusually shielded (δMe 1 04, δH-19 5 1) A one-proton doublet located at δ5 2 (J = 10 5 Hz) was reminiscent of the oxazine H-17 proton of the strychnobiline type alkaloids [6–9] as well as that of geissospermine [10] Multiple irradiation experiments were performed to establish interproton connections and partial structure **A** was deduced Such a structure suggested the presence of an isoretuline moiety in **6**

The protons, which were not involved in substructure A could be placed into two CH_2–CH_2 units, two CH_3–CH=C–CH_2 units and one CH–CH_2–CH unit Although these fragments could be accomodated into two retuline/isoretuline subunits (hypothesis I) or into two isoretuline/geissoschizine subunits (hypothesis II), the best fit was obtained for a strychnobiline type structure (hypothesis I) As the MW of strychnobiline of *S variabilis* [6] is 614, compound **6** could be a strychnobiline with a supplementary single or double bond Observation of a one proton singlet (δ6 2, $W_{1/2}$ = 2 Hz) in the ^{1}H NMR spectrum of **6** led us to propose a dehydro-16,17 isostrychnobiline structure for this compound The location of the new bond rests on the observation of a singlet at δ3 73 (H-2) and on the appearance of H-15 as a triplet, the chemical shift of H-17 is normal for an enol ether structure Comparison of the NMR spectrum of **6** with the data published for 12′-OH isostrychnobiline [8] and for isostrychnobiline [6] shows that **6** belongs to the *iso* series Configuration of the oxazinic C(17′) and conformation around the C-16–C-17′ bond cannot be determined with certainty as it was for 12′-OH isostrychnobiline [8] The deshielding of H-12′ (δ7 82) is indicative of a conformation around the N-1–CO bond in which the oxygen of the carbonyl faces H-12′, *ca* 10% of the other rotamer appears in the ^{1}H NMR spectrum Using the analysis of the ^{1}H NMR parameters published by Tavernier *et al* [11] for the retuline–isoretuline system, one may build a molecular stereomodel of **6** in which the D ring of the dehydro moiety is in a boat form (broad Me-18′ resonance, large vicinal coupling constant for CH_2-21′) In this particular conformation Me-18′ lies on top of the C-16′–C-17′ double bond in its shielding area [12]

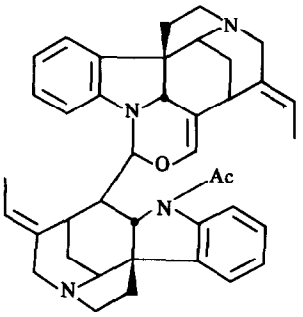
The mass spectrum of **6** displays the ubiquitous m/z 121, 122 ions of ethyl piperideines and m/z 130, 144 ions of tryptamine In the middle range of the mass spectrum, a cluster of ions at m/z 305, 306 and 307 is due to the cleavage of the C-17′–C-16′ bond as was found in strychnobiline [6] Alternative cleavages of the oxazine bonds with hydrogen migrations might yield the fragments at m/z 293, 309, 310 and 311 A noteworthy fragment is also found at m/z 583 $[M-29]^+$ and in the absence of an aldehyde, it may be explained by a double bond migration in the oxazoline ring followed by expulsion of the C-17 H–O fragment Other structures such as **6′**



A

Table 1 ^{1}H NMR spectrum of alkaloid **6**

	δ (ppm)	Multiplicity	J (Hz)		δ (ppm)	Multiplicity	J (Hz)
H-2	3 73	*s*	$W_{1/2}$ = 3	H-2′	4 23	*d*	10 5
H-3	3 82	*s*	$W_{1/2}$ = 7	H-3′	3 56	*t*	
H-14$_A$	2 21	*dt*	15, 3 5, 3 5	H-12′	7 82	*d*	7 5
H-14$_B$	1 33	*br d*	15	H-14′$_A$	2 02		
H-15	2 82	*br s*		H-14′$_B$	1 64	*dt*	15, 3, 3
H-17	6 20	*s*	$W_{1/2}$ = 2	H-15′	3 1	*q*	3
H-18	1 04	*br d*	7	H-16′	2 52	*dt*	3, 10 5
H-19	5 10	*br q*	7	H-17′	5 20	*d*	10 5
H-21$_A$	3 7	*br d*	14 5	H-18′	1 68	*dd*	7, 2
H-21$_B$	2 6	*d*	14 5	H-19′	5 66	*q*	7
				H-21′$_A$	3 7	*d*	16
				H-21′$_B$	3 25	*br d*	16



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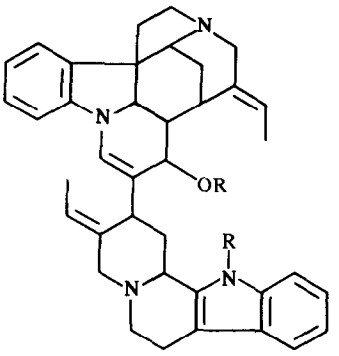


6' R or R¹ = Ac

of the longicaudatine type [2] are ruled out by the non-conformity of the IR and mass spectrum of **6** with what would be expected for **6'**

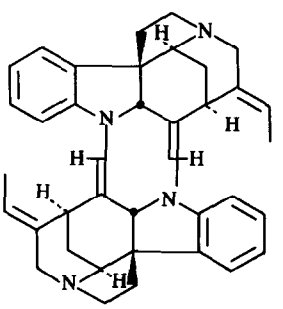
The order of elution of the root bark alkaloids is matopensine **5**, isoretulinal **1**, dehydroisostrychnobiline **6**, desacetyl isoretuline **3**, desacetylretuline **4** and retuline **2**

The alkaloid content of the stem bark was somewhat lower (4 7 g/kg) and seemed more complex due to the presence of several interconverting retuline type alkaloids Dehydroisostrychnobiline **6** was not found in the stem bark, matopensine **5**, however, was present, accompanied by its mono-*N*-oxide **7** and by the parent nordihydrotoxiferine **8** The ^{1}H NMR spectrum of the *N*-oxide of matopensine was complex due to its now unsymmetrical structure, large chemical shift differences were found for protons in the vicinity of the *N*-oxide group (H-3, H-21, H-19, Me-18) as well as for remote protons such as the aromatics or the H-2 (see Experimental for data) This fact might be attributed to conformational changes within the *N*-oxide bearing moiety This structure was unambiguously proved by preparation of **7** from **6** (MCPBA, CH_2Cl_2)

Along with these dimeric alkaloids, we found six alkaloids related to isoretuline isoretuline **9**, *N*(1)-desacetyl-18-acetoxy isoretuline **10** [2], Wieland-Gumlich aldehyde **11**, its diol **12**, desoxy Wieland-Gumlich aldehyde **13**—the desacetyl counterpart of compound **1** of the rootbark—and 11-methoxy isoretuline **15** The first five compounds were identified by direct comparison with authentic samples and also by their physical and spectral properties The retuline series was represented by retuline **2** by its *O*-acetyl derivative **14** and by 11-methoxy retuline **16**, all gave ^{1}H NMR spectra complicated by the presence of rotamers [11, 13]

11-Methoxy isoretuline **15** was found in fractions containing isoretuline **9** Its ^{1}H NMR spectrum was similar to that of isoretuline [11] except for a three proton singlet at δ3 82 and for an AMX pattern in the aromatic area (J_{AM} = 8 Hz, J_{MX} < 2 Hz) These data were indicative of the presence of a methoxyl substituent at position C-10 or C-11, on the benzene ring Confirmation of this hypothesis was obtained through the mass spectrum of **15**, which showed similarities with that of retuline except for the fragments containing the indole rings (*m/z* 160, 174, 216, 323 and 368) [14] Final location of the methoxyl group in C-11 was based on the ^{13}C NMR spectrum of **15**, which was interpreted according to Wenkert's results on isoretuline [15] Introduction of a methoxyl group on the C-10 of **9** would have left almost unchanged the chemical shift of C-12 (δ117), the absence of any methine in this area (δ_{CH} 122 7, 109 8, 105 5) did not favour this hypothesis and this is the reason why we propose structure 11-methoxy isoretuline for this novel alkaloid

The retuline containing fractions presented different colors, from pale orange to purple, upon spraying with Ce(IV) spray Whereas the 'orange' fractions were pure retuline **2** as demonstrated by 400 MHz ^{1}H NMR, the 'purple' fractions were 1 1 mixtures of retuline **2** and of a methoxy retuline **16** The high field part of the ^{1}H NMR spectrum was superimposable on the complex retuline spectrum (mixture of rotamers) except for two singlets at δ3 8 and 3 83 (3 1 ratio) In the aromatic part of the



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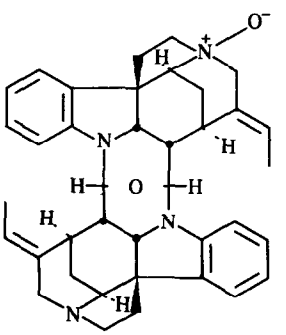

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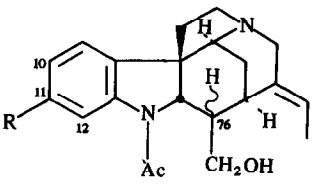

	R	
2	H	16 βH
15	OMe	16 βH
9	H	16 αH
16	OMe	16 αH

spectrum, a broad singlet at δ7 78 ($W_{1/2}$ = 4 Hz) corresponds to H-12 of the methoxyretuline rotamer in which the carbonyl oxygen is oriented towards C-13 We therefore conclude that **16** is 11-methoxy retuline, although we do not claim its isolation as pure material

Besides these '*Strychnos*' type alkaloids, small quantities of 16*R*-isositsirikine **17** were found (< 1% of AM), this alkaloid was identified by a synthesis from geissoschizine and by comparison of the 400 MHz ^{1}H NMR spectrum of **17** with literature data [16]

The order of elution of the alkaloids in the stem bark is matopensine **5**, nordihydrotoxiferine **8**, 16*R*-isositsirikine **17**, isoretuline **9**, 11-methoxyisoretuline **15**, N(1)-desacetyl-18-acetoxy isoretuline **10**, *O*-acetylretuline **14**, matopensine *N*-oxide **7**, retuline **2**, 11-methoxy retuline **16**, Wieland-Gumlich aldehyde **11** and diol **13**

The leaves AM, which was available in low yield, presented three major spots on TLC Prep TLC yielded three alkaloids identified as *O*-acetyl retuline **14** (3% of AM), *O*-acetyl isoretuline **18** (13% of AM) and retuline **2** (10% of AM) by comparison with authentic samples

Eighteen alkaloids have been identified from the root bark, stem bark and leaves of *Strychnos kasengaensis* They all belong to the akuammicine type of alkaloids except 16*R*-isositsirikine **17** Although rare, this kind of skeleton is found, among *Strychnos* species, in dimers such as geissospermine [10] and longicaudatine [2] or in quaternary ammonium salts such as the diplocelines [17] The three dimers dehydro isostrychnobiline **6**, matopensine **5** and nor-dihydrotoxiferine **8** represent different stages of the coupling between monomers such as desoxy Wieland-Gumlich aldehyde All other monomers result from epimerization at C-16, reduction and acetylations of the parent aldehydes

EXPERIMENTAL

General Plant material was collected by one of us (C D) in Zaire and identified by H Breyne Plant material was collected under the 'Etude phytochimique de la flore du Zaire' research project A herbarium specimen is deposited in the Brussels National Gardens under No HB 4465 Mps are uncorr ^{1}H NMR were measured at 400 MHz with a prototype built at the Institut d'Electronique Fondamentale, Université de Paris-Sud, Orsay ^{13}C NMR was obtained at 15 MHz or at 22 5 MHz

Typical extraction procedure Finely ground root bark (280 g) was wetted with 160 ml of conc NH_4OH half diluted in H_2O and lixiviated overnight in 20 l of EtOAc Completeness of the extraction is verified by the Valser Mayer test The organic soln was extracted with a 2% H_2SO_4, the acid layer separated, made alkaline with NH_4OH and extracted with $CHCl_3$ The $CHCl_3$ soln was washed with H_2O, dried (Na_2SO_4) and evapd *in vacuo* We obtained 3 88 g of crude AM, yield 13 8 g/kg In a similar fashion 880 g of milled stem bark yielded 5 3 g of AM (6 0 g/kg) and 460 g of ground leaves yielded 0 6 g of AM (1 3 g/kg)

Typical separation procedure The AM from the stem bark (5 3 g) was fractionated on 200 g Merck silica gel H-60 (elution pressure 10 bar) After a 200 ml dead volume', 30 ml fractions were collected Solvents were $CHCl_3$ (fr 1–80) and mixtures of $CHCl_3$–MeOH (99 1) (fr 81–182), (49 1) (fr 183–253), (19 1) (fr 254–338), (9 1) (fr 339–427), (4 1) (fr 428–528), (1 1) (fr 529–600) Matopensine **5**, its mono-*N*-oxide **7**, nor dihydrotoxiferin **8**, isositsirikine **17**, were in fractions 178–237, N(1)-desacetyl-18-acetoxy isoretuline **10** was in fr 262–272 along with isoretuline **9**, 11-OMe isoretuline **15** and O-acetylretuline **14**, retuline **2** and 11-OMe retuline **16** were in fr 329–346, Wieland-Gumlich aldehyde **11**, diol **13** and desoxy **1** Wieland-Gumlich aldehyde **13** were in fr 466–522 Analytical samples were obtained by prep TLC of these fractions Evaluation of the relative percentages is as follows **5** (1%), **7** (< 1%), **8** (1 5%), **17** (< 1%), **10** (1 5%), **9** + **15** (2%), **14** (0 7%), **2** (25%), **16** (5%), **13** (8%), **11** (1 5%), **13** (6%)

New alkaloids Matopensine **5** (CR red), $[\alpha]_D$ = +105° (*c* 0 6, MeOH); UV λ_{max}^{EtOH} nm 217 (log ε 4 46), 292 (3 92), 315 (3 95), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1650, 1605, 1480, 1460, 1390, 1300, 1270, MS *m/z* (rel int) 570 3366 ($C_{38}H_{42}N_4O$, 45%), 412 (2), 322 (10), 307 (15), 279 (45), 277 (75), 180 (30), 144 (100), 130 (40), 122 (35), 121 (30), ^{1}H NMR see ref [5]

16,17-*Dehydroisostrychnobiline* **6** (CR purple), $[\alpha]_D$ = +49° (*c* 0 64, MeOH), UV λ_{max}^{MeOH} nm 218 (log ε 4 38), 250 (4 19), 280 (3 75), 290 (3 75), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1660, 1650, 1600, 1470, 1450, 1385, MS *m/z* (rel int) 612 (50), 583 (20), 555 (5), 515 (2), 321, 320, 319, 305 (40), 293 (60), 144 (100), 130 (30), 122 (20), 121 (30), ^{1}H NMR see Table 1

*Matopensine mono-*N*-oxide* **7** (CR red), UV λ_{max}^{MeOH} 217, 263, 313, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1650 (weak), 1600, 1485, 1460, 1390, 1270; MS *m/z* (rel int) 586 (5), 584 (5), 570 (60), 569 (40), 568 (80), 552 (10), 305 (30), 293 (35), 279 (50), 278 (40), 277 (95), 275 (50), 180 (20), 164 (50), 144 (100), 130 (40), 122 (50), 121 (60), ^{1}H NMR (400 MHz, $CDCl_3$) δ7 2–7 1 (*m*, 4H), 6 81 (*t*, *J* = 7 Hz, 1H), 6 76 (*t*, *J* = 7 Hz, 1H), 6 23 (*d*, *J* = 7 Hz, 1H), 6 16 (*d*, *J* = 7 Hz, 1H), 5 65 (*br*, *q*, *J* = 7 Hz, 1H), 5 36 (*br q*, *J* = 7 Hz, 1H), 5 32 (*br s*, 2H), 4 2 (*d*, *J* = 5 Hz, 1H), 4 13 (*d*, *J* = 13 Hz, 1H), 4 07 (*d*, *J* = 7 Hz, 1H), 3 64 (*br s*, 1H), 3 1 (*br s*, 1H), 2 97 (*br s*, 1H), 1 9 (*br d*, *J* = 7 Hz, 3H), 1 83 (*br d*, *J* = 7 Hz, 3H)

Isoretulinal **1** (Supplementary data, for other properties see ref [3]) Mp 188–191, ^{13}C NMR ($CDCl_3$) 203 0 (*d*, C-17), 168 8 (*s*, $\underline{C}OCH_3$), 140 2 (*s*, C-13), 138 1 (*s*, C-8), 133 2 (*s*, C-20), 128 0 (*d*, C-11), 124 7 (*d*, C-10), 122 7 (*d*, C-9), 121 6 (*d*, C-19), 115 6 (*d*, C-12), 67 2 (*d*, C-2), 61 8 (*d*, C-3), 58 0 (*t*, C-21), 57 5 (*d*, C-16), 54 1 (*t*, C-5), 50 8 (*s*, C-7), 43 9 (*t*, C-6), 30 3 (*d*, C-15), 27 4 (*t*, C-14), 23 9 (*q*, $\underline{C}H_3CO$), 12 7 (*q*, C-18)

11-*Methoxy isoretuline* **15** (CR pink), $[\alpha]_D$ = +145° (*c* 0 2, MeOH), UV λ_{max}^{MeOH} (log ε) 223 (4 32), 250 (4 05), 292 (3 69) nm, IR ν_{max}^{KBr} cm^{-1} 3400, 1660, 1610, 1595, 1390, 1040; MS *m/z* (rel int) 368 (50), 353 (10), 323 (45), 216 (5), 174 (40), 166 (50), 160 (10), 144 (10), 121 (100), ^{1}H NMR (400 MHz, $CDCl_3$) 7 07 (*br d*, *J* = 8 Hz), 6 7 (*br s*), 6 67 (*d*, *J* = 8 Hz), 3 82 (*s*, 3H), other peaks as in isoretuline, ^{13}C NMR (22 5 MHz, $CDCl_3$) δ170 6 (C=O), 159 7 (C-11), 143 0 (C-13), 122 7 (C-9), 120 6 (C-19), 109 8 (C-10), 105 5 (C-12), 70 7 (C-2), 64 8 (C-17), 61 8 (C-3), 58 9 (C-21), 55 8 (OMe), 53 9 (C-5), 52 1 (C-7), 47 7 (C-16), 42 6 (C-6), 31 7 (C-15), 28 4 (C-14), 23 2 ($CO\underline{C}H_3$), 12 8 (C-18), high resolution MS 368 2018 ($C_{22}H_{28}N_2O_3$), 323 1791 ($C_{20}H_{23}N_2O_2$)

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have been informed of the simultaneous isolation and structure determination of dehydroisostrychnobiline [18] We thank Professor L Angenot for indicating this reference and for an exchange of information We thank Dr S K Kan (Université d'Orsay) for 400 MHz ^{1}H NMR spectra and Mr Merle (ICI Pharma) and H Baillia for ^{13}C NMR spectra We gratefully acknowledge support by 'Ministère de la coopération au développement' de Belgique

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