(10 ml). The mixture was refluxed under N_2 for 5 hr. The soln was then filtered, the residue washed with MeOH and the filtrates combined with the washings and concd. It was then partitioned between an ice-cold aq. NH_3 soln and $CHCl_3$. The $CHCl_3$ extract was washed (H_2O), dried (Na_2SO_4) and evapd to give 2,16-dihydroscholaricine as a gum (1 mg) which was then purified by prep. TLC on silica gel using MeOH–$CHCl_3$–NH_3 (1:9:0.1); UV λ_{max}^{MeOH}: 245, 290 nm; λ_{min}: 257 nm; MS: *m/z* 358.1885, (calc. for $C_{20}H_{26}N_2O_4$ 358.1892), 286, 257, 212, 160, 146, 129, 97.

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THREE QUINICINE DERIVED ALKALOIDS FROM *GUETTARDA TRIMERA**

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Key Word Index—*Guettarda trimera*; Rubiaceae; trunk bark; alkaloids; quinicine derivatives.

Abstract—Three alkaloids, isolated from the trunk bark of *Guettarda trimera* have been identified as derivatives of quinicine on the basis of mass spectrometry, ^1H and ^{13}C NMR data.

INTRODUCTION

Extracts of *Guettarda* species have been extensively studied in our laboratory as they represent an interesting source of biosynthetic intermediates in the indole alkaloid series [1]. Moreover, it appears that each species produces alkaloids of only one structural type. This unusual situation is important for the chemotaxonomic classification of the Rubiaceae.

We wish now to report the isolation of three quinicine derivatives **1**, **3** and **4** from the trunk bark of *G. trimera*. This type of alkaloid was previously prepared by partial synthesis from quinine but was not known as natural products.

RESULTS AND DISCUSSION

Air dried bark of *G. trimera* was powdered and extracted following a classical method. Alkaloid **1** was obtained directly from the crude extract by crystallization from hot acetone. Alkaloids **3** and **4** were subsequently isolated by CC of the mother liquors on alumina and prep. TLC separation of alkaloid enriched fractions on silica gel.

Compound **1**, colourless crystals (mp 156°; acetone), $[\alpha]_D^{20} - 84°$ ($CHCl_3$, *c* 1.2), $[M]^+$ at *m/z* 342, displayed a UV spectrum identical to that of quinine (λ_{max}^{EtOH} 233, 271, 281, 291, 323, 333). Fragments were observed in the mass spectrum at *m/z* 189 and 160 which suggested a methoxyquinoline derivative bearing a secondary hydroxyl group at C-9. However the fragments at *m/z* 138, 110 and 82 characteristic of the quinuclidine ring of quinine were absent.

In the ^1H NMR spectrum of **1** resonances were observed for a N–Me group (*s*, δ2.2) and a methoxyl group (*s*, δ3.9), the terminal methyl hydrogens of an ethyl side chain (*t*, 3H, δ0.85) and five aromatic protons. The signal at δ5.32 in **1** was observed to shift downfield to 6.41 in its

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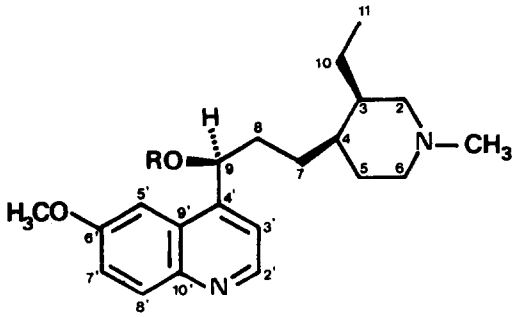
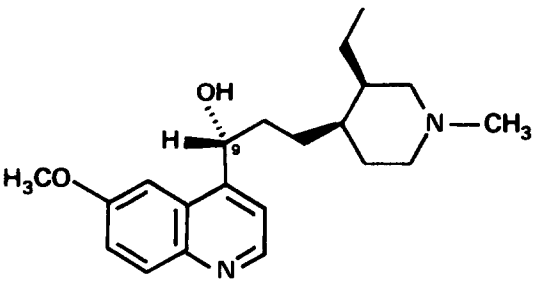
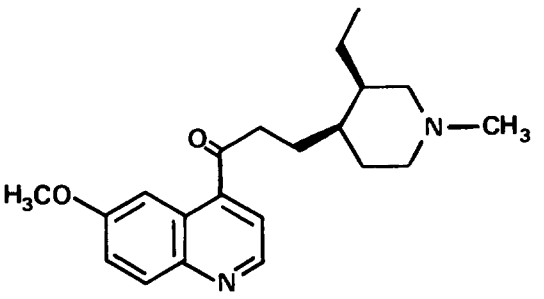


1 R=H, N-methyl dihydroquinicinol
2 R=Ac



3 9-epi-N-methyl dihydroquinicinol



4 N-methyl dihydroquinicine

O-acetyl derivative **2** providing a means of identifying the signal produced by the methine hydrogen geminal to the secondary hydroxyl group. Furthermore the appearance of this signal as a doublet of doublets coupled to two adjacent methylene protons rather than a doublet as in quinine suggested that **1** was related to members of the dihydroquinicinol series. This was further supported by examination of the ^{13}C NMR spectra of compounds **1** and **2** (see Table 1). Finally, **1** was proved to be identical with *N*-methyl dihydroquinicinol which was prepared by methylation of dihydroquinicinol* on reaction with formaldehyde and sodium borohydride at pH 4.

The second alkaloid **3** [colourless crystals, mp 164°, acetone, $[\alpha]_D^{20} + 58°$ ($CHCl_3$, *c* 0.3)] proved to be isomeric with **1**. The mass spectra of **1** and **3** were almost identical and striking resemblances were noted in their ^1H NMR spectra with the exception of the signal for the C-9 hydrogen resonance. Definitive proof that these alkaloids are epimeric alcohols was obtained by chemical correlation of **3** with 9-epi-*N*-methyl dihydroquinicinol* obtained by methylation of the corresponding secondary amino derivative.

The third alkaloid [amorphous, $[\alpha]_D^{20} - 3°$ ($CHCl_3$, *c* 1), $[M]^+$ *m/z* 340] possessed a carbonyl absorption at 1690 cm^{-1} in its IR spectrum and showed UV absorption at λ_{max}^{EtOH} 222, 245, 262, 300, 356 nm. From these data and ^1H and ^{13}C NMR data structure **4** was proposed for this compound. As expected a mixture of compounds **1** and **3** was obtained on reduction of **4** with sodium borohydride.

The isolation of the quinicinol type alkaloids **1** and **3** is interesting from the biogenetic point of view. Indeed quinicine, the only known natural product in this series has been previously isolated in traces quantities from the

Table 1. ^{13}C NMR chemical shift values of alkaloids **1–4***

Carbon	1	2	3	4
2	57.8	57.9	58	58
3	40	39.8	40.2	40.3
4	36.7	37	37.2	37.1
5	28.1	28.1	28.4	28.2
6	54.1	54.6	54.6	54.5
7	26	26	26.5	24.5
8	35.8	33.1	35.8	40
9	70.1	72	70.9	204
10	20	19.5	19.8	19.8
11	12	12.1	12.2	12.2
NMe	46.5	46.6	46.6	46.6
OMe	55.3	55.5	55.6	55.5
$\underline{C}H_3$–C=O		21.1		
CH_3–$\underline{C}$=O		170		

*Recorded in $CDCl_3$ (δ, TMS). All assignments were supported by OR experiments

*Samples given by Mr. Gueremy, Pharmuka, 92231 Gennevilliers, France, whom we thank.

trunk bark of *Cinchona* [2] and could possibly be an artefact since this alkaloid can be produced from quinine by heating in an acid medium.

The formation of alkaloids **1** and **3**, isolated as major products in *G. trimera* demands that two supplementary steps occur in their biogenesis from quinine: (1) methylation of the quinuclidine nitrogen which facilitates also the hydride transfert leading to quinicine derivatives [2] and (2) reduction of the ketone function of **4** into **1** and **3**.

EXPERIMENTAL

UV spectra were run in EtOH and ^{1}H NMR spectra were recorded in $CDCl_3$ (TMS as int standard) at 400 MHz. ^{13}C NMR spectra were measured in $CDCl_3$ at 22.63 MHz.

Plant material was collected by two of us (J. Pusset and G. Chauviere) at Mont Kaala in March 1981. A herbarium specimen is kept under reference Pusset–Chauviere N° 16 at the Centre ORSTOM in Nouméa (New Caledonia).

Isolation of alkaloids. Extraction of dry trunk bark (5 kg) of *G. trimera* in the classical manner gave 16.6 g of crude alkaloids. By crystallization of the crude extract from Me_2CO pure alkaloid **1**, mp 156° (8.16 g) was obtained. The mother liquors were subsequently purified by CC on alumina followed by prep. TLC on silica gel. In this manner alkaloids **3** (0.15 g), mp 164° (Me_2CO), and **4** (0.25 g), amorphous, were isolated.

N-*Methyl dihydroquinicinol* (**1**). Alkaloid **1** crystallized from Me_2CO, mp 156°, $[\alpha]_D^{20}$ −84° ($CHCl_3$, *c* 1.2). It exhibited the following spectral properties, UV λ_{max}^{EtOH} nm: 233, 271, 281, 291, 323, 333; MS *m/z* (rel. int.): 342 $[M]^+$ (50), 327 $[M-Me]^+$ (27), 325 (5), 324 (4), 313 $[M-Et]^+$ (3), 295 (3), 270 (3), 202 (7), 189 (50), 160 (57), 154 (100); ^{1}H NMR: δ0.85 (*t*, C$\underline{H}_3$–CH_2), 2.2 (*s*, NMe), 3.9 (*s*, OMe), 5.32 (*dd*, J = 8 Hz, J' = 5 Hz, H-9), 7.2 (*d*, J = 3 Hz, H-5'), 7.35 (*dd*, J = 9 Hz, J' = 3 Hz, H-7'), 7.5 (*d*, J = 4.5 Hz, H-3'), 8 (*d*, J = 9 Hz, H-8'), 8.68 (*d*, J = 4.5 Hz, H-2'); for ^{13}C NMR see Table 1.

Acetylation. Alkaloid **1** (50 mg) was reacted with Ac_2O in pyridine (1 ml) at room temp for 24 hr. After usual work-up and purification the pure O-acetyl derivative **2** was obtained: MS *m/z* 384; IR ν_{max}^{nujol} cm^{-1}: 1730 (acetyl); ^{1}H NMR: δ2.21 (*s*, Ac), 6.41 (*t*, H-9).

9-*Epi*-N-*methyl-dihydroquinicinol* (**3**). Alkaloid **3** crystallized from Me_2CO, mp 164°, $[\alpha]_D^{20}$ + 58° ($CHCl_3$, *c* 0.3). It exhibited spectral properties very similar to those of **1** except for the H-9 signal in the ^{1}H NMR spectrum (δ5.30, *dd*, J = 8 Hz, J' = 4 Hz). For ^{13}C NMR see Table 1.

N-*Methyl dihydroquinicine* (**4**). Alkaloid **4** is amorphous; $[\alpha]_D^{20}$ − 3° ($CHCl_3$, *c* 1). It exhibited the following spectral properties, UV λ_{max}^{EtOH} nm: 222, 245, 262, 300, 356; IR ν_{max}^{nujol} cm^{-1}: 1690 (conjugated ketone); MS *m/z* 340 $[M]^+$ (64), 325 (3), 324 (14), 153 (68), 123 (100); ^{1}H NMR: δ0.9 (*t*, C$\underline{H}_3$–CH_2), 2.2 (*s*, NMe), 3.95 (*s*, OMe), 7.42 (*dd*, J = 9 Hz, J' = 3 Hz, H-7'), 7.6 (*d*, J = 4 Hz, H-5'), 7.8 (*d*, J = 3 Hz, H-3'), 8.05 (*d*, J = 9 Hz, H-8'), 8.87 (*d*, J = 4 Hz, H-2'). For ^{13}C NMR see Table 1.

Methylation of dihydroquinicinol and its C-9 *epimer.* Dihydroquinicinols (50 mg) were dissolved in an aq. soln of HCHO (10 ml) at pH 4 (HOAc). The soln was then treated at 0° with an excess of $NaBH_4$ for 30 min. The methylated products were isolated in the usual manner and proved to be identical to the natural products **1** and **3**.

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