Prenylated Flavanones from *Monotes engleri*: On-line Structure Elucidation by LC/UV/NMR

by **Eliane Garo**, **Jean-Luc Wolfender**, and **Kurt Hostettmann***

Institut de Pharmacognosie et Phytochimie, Université de Lausanne, BEP, CH-1015 Lausanne

and **Wolf Hiller**

Varian GmbH, Alsfelder Strasse 3, D-64289 Darmstadt

and **Sándor Antus**

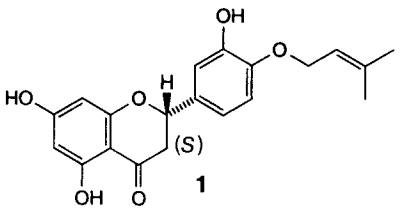
Lehrstuhl für Organische Chemie der Kossuth-Universität, Postfach 20, H-4010 Debrecen

and **Steven Mavi**

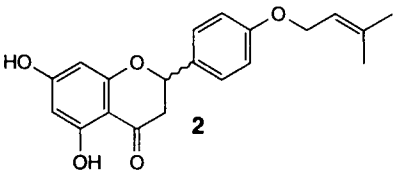
National Herbarium and Botanic Garden, P.O. Box 8100, Causeway, Harare, Zimbabwe

The CH_2Cl_2 extract of *Monotes engleri* GILG. (Dipterocarpaceae) showed antifungal activity against the yeast *Candida albicans* in our bioautographic TLC assays. After a first fractionation of the crude extract, the bioactivity was located in one of the fractions. To perform an efficient targeted isolation of the active compounds, LC/UV/MS and LC/UV/NMR analyses of the crude extract and the active fraction were performed. LC/UV, LC/MS, and LC/NMR data (1D and 2D) allowed the identification of **1** as (2S)-*2,3-dihydro-5,7-dihydroxy-2-{3-hydroxy-4-[(3-methylbut-2-enyl)oxy]phenyl}-4*H-*1-benzopyran-4-one*, a new prenylated flavanone, named monotesone A. Subsequent isolation of **1** has permitted the determination of its absolute configuration on the basis of CD measurements. Three other prenylated flavanones **2**–**4** were isolated from the same extract. Compound **3** was identified as *2-(3,5-dihydroxyphenyl)-2,3-dihydro-5,7-dihydroxy-6,8-bis(3-methylbut-2-enyl)-4*H-*1-benzopyran-4-one*, another new natural product, named monotesone B. The structures of **2** and **4** were established as selinone and lonchocarpol A, respectively. The antifungal activity against *Candida albicans* was determined for all compounds.

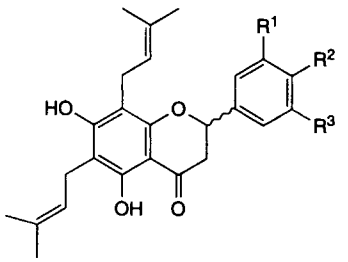
1. Introduction. – In the course of our phytochemical investigations of plants used in traditional medicine in Zimbabwe, we have studied the Dipterocarpaceae *Monotes engleri* GILG. The leaves of this tree are used by healers to wash and rub the skin of the lepers [1]. In a series of preliminary screenings, the CH_2Cl_2 extract of the leaves of *M. engleri* displayed an interesting activity against the growth of the yeast *Candida albicans* in a bioautographic TLC assay [2], while the MeOH extract was found inactive. To obtain preliminary structural information on the active constituents, an LC/UV/MS analysis of the active extract was carried out with a $MeCN/H_2O$ gradient on a reversed-phase column. The four main constituents (**1**–**4**) showed typical UV spectra of flavanones (λ_{max} 330 (sh), 290 nm) [3]. Prenylated flavanones were already reported from this plant [4]. To identify **1**–**4** and to avoid the useless isolation of already known and well-established compounds, an LC/NMR analysis has been performed. In this paper, the potential of LC/UV, LC/MS, and LC/NMR for a full on-line identification of flavanones is discussed.



1



2



3 R^1=R^3=OH; R^2=H
4 R^1=R^3=H; R^2=OH

2. Results. – Dried and powdered leaves of *Monotes engleri* were successively extracted at room temperature with CH_2Cl_2 and MeOH. Both extracts were submitted to bioautographic TLC assays against the yeast *Candida albicans* [2] and the phytopathogenic fungus *Cladosporium cucumerinum* [5]. The CH_2Cl_2 extract displayed only antifungal activity against *C. albicans*. This activity was linked to a compound, causing a strong UV absorbing spot on TLC at 254 nm, which most probably corresponded, in the LC/UV analysis at 254 nm, to the main compound **1** of the extract. An LC/MS analysis of the extract using a thermospray interface (TSP) was carried out, and flavanones **1**–**4** showed molecular ions $[M + H]^+$ at *m/z* 357, 341, 425, and 409, respectively. A different mass-fragmentation pattern was observed for **1**–**4**. Compounds **1** and **2** exhibited a loss of 68 amu ($[M + H - C_4H_8]^+$), characteristic for an *O*-prenyl substituent [6]. For **3** and **4**, a loss of 55 amu was visible, attributable to a *C*-prenyl group. A second fragment, in both cases at *m/z* 279, should correspond to a loss of a second prenyl unit associated with different dehydrations. Based on these findings and according to the molecular weight of a flavanone skeleton, **1** and **2** should have one *O*-prenyl and three and two OH substituents, respectively. On the other hand, **3** and **4** should be substituted by two *C*-prenyl units and four and three OH groups, respectively. Since no product with the same molecular weight as **1** (Mol. Wt. 356) has yet been described in the plant, a detailed investigation was then undertaken.

To obtain more structural information, a LC/UV/^{1}H-NMR analysis of the crude extract was performed. The same LC conditions as for LC/UV/MS were applied, except that, in this case, a MeCN/D_2O instead of MeCN/H_2O gradient was used. The use of D_2O permitted to lock the NMR spectrometer on deuterium, and a sharper line for the residual signal of water was obtained, allowing a more efficient solvent suppression. To remove the solvent signal of MeCN and its two ^{13}C satellites, as well as the residual HOD resonance, a fast solvent-suppression sequence WET [7] was run before each acquisition. This sequence consisted of a combination of laminar shifted shaped selective pulses associated with dephasing gradients and selective ^{13}C decoupling. For the on-flow

LC/NMR analysis, 1 mg of the CH_2Cl_2 extract of *M. engleri* was injected into the column. As shown in *Fig. 1*, two compounds, **1** and **2**, with a t_R of 26.3 min and 31.6 min, respectively, were detected. The other constituents occurring in lower amounts could not be observed. The two on-flow LC/^{1}H-NMR spectra of **1** and **2** were quite similar (*Fig. 2*); the only difference between them was found in the aromatic-proton region. The signal/noise ratio of the different resonances was rather weak, but a careful analysis of these on-flow spectra permitted to obtain different structural information. For **1**, the presence of a flavanone skeleton with a prenyl unit could be established: two CH_2 protons at 2.9 and 3.2 ppm suggested the presence of a γ-dihydropyrone ring. The deshielded *doublet* for the CH_2 group of the prenyl moiety at 4.7 ppm confirmed the O-connection of the prenyl unit, as deduced from the LC/MS data. In the ^{1}H-NMR spectrum of **2**, there was an additional signal at 7.5 ppm when compared with the spectrum of **1**. This suggested a different substitution pattern on the B-ring. These ^{1}H-NMR spectra were, however, not sufficient for a complete identification of **1** and **2**. Fractionation of the crude extract by MPLC on SiO_2 was then undertaken to afford 13 fractions (*I–XIII*). The activity was reproduced in fraction *X* which was analyzed by stop-flow LC/NMR. Due to the enrichment obtained after fractionation and the use of the stop-flow technique, the LC/NMR quality of the spectrum of **1** was strongly improved compared to the on-flow results. Furthermore, the propiononitrile impurities, which cause important accompanying signals in the on-flow measurement, were efficiently eliminated by using selective shaped pulses allowing the simultaneous suppression of the four solvent lines. In the stop-flow mode, as the analyte can be kept indefinitely in the LC/NMR cell, different 1D- and 2D-NMR correlation experiments were performed: GHSQC (gradient heteronuclear single quantum coherence), GHMBC (gradient heteronuclear multiple quantum coherence),

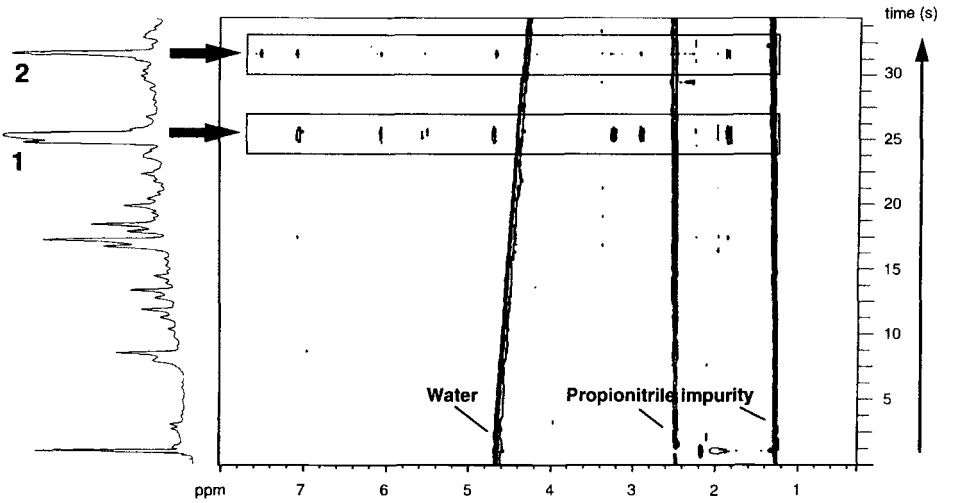


Fig. 1. *2D-LC/^{1}H-NMR Chromatogram of the crude CH_2Cl_2 extract of* M. engleri (Dipterocarpaceae). The two main compounds are recorded by LC/^{1}H-NMR: **1** at 26.3 min and **2** at 31.6 min. HPLC: *Novapak* C_{18} column (4 mm, 150 × 3.9 mm i.d.); gradient: MeCN/D_2O 20:80 → 70:30 in 50 min (1 ml/min); quantity injected: 1 mg; acquisition: 24 transients/step. LC/UV Chromatogram (vertical display) with corresponding UV spectra recorded on-line. The shift of the HOD line is due to the change in composition during the LC/gradient.

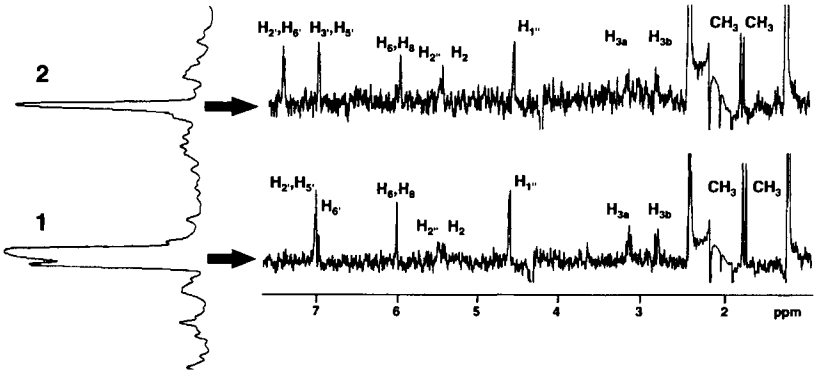


Fig. 2. *LC/^{1}H-NMR of the two main compounds recorded on-line in the CH_2Cl_2 extract of* M. engleri. The Ar–CH(O)–CH_2–CO chain of the flavanone skeleton and the prenyl unit are visible in both spectra.

1D-TOCSY, and WET-NOESY (2D nuclear *Overhauser* effect) NMR experiments were recorded in a total acquisition time of 9.6 h.

In the stop-flow LC/^{1}H-NMR spectrum the presence of the *O*-prenylated flavanone could be clearly confirmed (*Figs. 3* and *4*). In the 1D-TOCSY, the connections between the prenyl protons were clearly shown. The GHSQC (*Fig. 5*) showed all C–H connectivities. In the GHMBC (*Fig. 6*), the long-range coupling of the γ-dihydropyrone ring were visible, and the position of the prenyl unit on the B-cycle could be determined. The combination of the data obtained with these two inverse ^{1}H,^{13}C correlation experiments permitted to assign all the ^{13}C resonances of **1** on-line directly in the LC eluent. To determine the exact position of the prenyl unit on the flavanone skeleton of **1**, a 2D WET-NOESY experiment was undertaken. This experiment allowed to observe different useful NOE correlations; however, the weak resolution of the H-pattern of the B-cycle in MeCN/D_2O did not permit to differentiate the H–C(2′) and H–C(5′), preventing the full structural assignment of **1**. A second stop-flow LC/NMR analysis of the active fraction *X* was then performed in MeOH/D_2O. In this latter solvent system, the three aromatic protons of the B-cycle could be observed at distinct chemical shifts. Two *doublets* (J = 7.8 Hz) at 6.90 and 6.95 ppm were attributable to the *ortho*-coupled protons H–C(6′) and H–C(5′), and one broad *singlet* at 6.97 ppm was due to H–C(2′) (*Table*). A second WET-NOESY was performed in this latter solvent system (*Fig. 4*). First, an irradiation of H–C(2) gave enhancement of the two aromatic protons, H–C(2′) and H–C(6′), at 6.97 and 6.90 ppm. A correlation with H–C(2) and 2 H–C(3) was also shown. Secondly, irradiation of H–C(1″) gave enhancement of the *ortho*-coupled proton at 6.95 ppm (H–C(5′)). Other NOE effects were also measured on the prenyl unit, particularly between H–C(1″) and H–C(2″), H–C(1″) and H–C(5″), as well as between H–C(2″) and H–C(4″). According to these observations, the attachement of the *O*-prenyl substituent at C(4′) was confirmed, and **1** could thus be identified on-line as *2,3-dihydro-5,7-dihydroxy-2-{3-hydroxy-4-[(3-methylbut-2-enyl)oxy]phenyl}-4*H*-1-benzopyran-4-one*, a new natural product, and its complete isolation was finally performed for the measurement of its physical data, as well as the determination of its absolute configuration. Pure compound **1** showed a negative optical rotation ($[\alpha]_D$ = – 24.7), and the absolute configuration at C(2) has been determined by CD experiments.

Table. *^{1}H-NMR Data of the LC Peak of* **1** *in* Fraction X *Recorded with Two Different Eluent Systems.* Coupling constants J in Hz

	MeOH/D_2O		MeCN/D_2O	
H-Atom	δ [ppm]	Multiplicity	δ [ppm]	Multiplicity
H–C(2′)	6.97	*s*	7.05	unresolved
H–C(5′)	6.95	*d* (J = 7.8)	7.05	unresolved
H–C(6′)	6.90	*d* (J = 7.8)	7.00	*d* (J = 8.8)
H–C(6)	5.95	*s*	6.06	*s*
H–C(8)	5.93	*s*	6.06	*s*
H–C(2″)	5.49	*t* (J = 6.4)	5.54	unresolved
H–C(2)	5.35	*dd* (J = 2.9, 12.2)	5.48	*dd* (J = 2.9, 12.7)
H–C(1″)	4.61	*d* (J = 6.8)	4.68	*d* (J = 6.8)
H_a–C(3)	3.11	*dd* (J = 12.2, 16.6)	3.22	*dd* (J = 12.7, 17.6)
H_b–C(3)	2.78	*dd* (J = 2.9, 16.6)	2.88	*dd* (J = 2.9, 17.6)
H–C(4″)	1.78	*s*	1.84	*s*
H–C(5″)	1.74	*s*	1.80	*s*

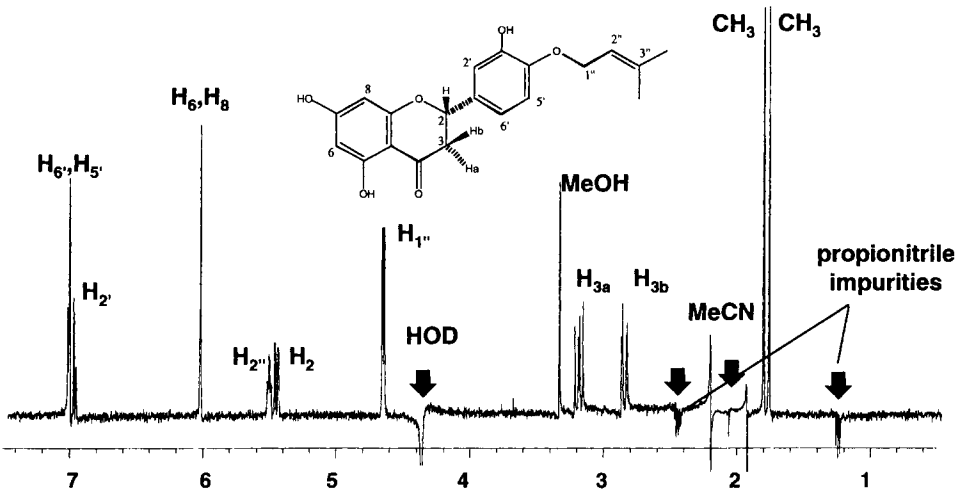


Fig. 3. *Stop-flow LC/^{1}H-NMR spectrum of* **1** *in the enriched fraction of* M. engleri *with multiple line supression with WET on MeCN and HOD, as well as on the propiononitrile impurities of MeCN.* Acquisition: 752 transients.

In the CD spectra of (–)-**1**, four *Cotton* effects were observed in MeOH (see *Exper. Part*). The positive effect at 328 nm and the negative one at 292 nm were connected with the n → π* and π → π* transitions of the aryl-ketone chromophore of the molecule, respectively. Since laevorotatory flavanones with equatorial 2-aryl substituents exhibit a positive *Cotton* effect due to the n → π* transitions (~330 nm) and a negative *Cotton* effect in the π → π* region (~ 280–290 nm) [8], and contrary to the *Cotton* effect of the benzene chromophore, the sign of the n → π* band of CD is independent on the substitution pattern of the aromatic ring system [9]; therefore, compound (–)-**1** possesses the absolute configuration (*S*) at C(2).

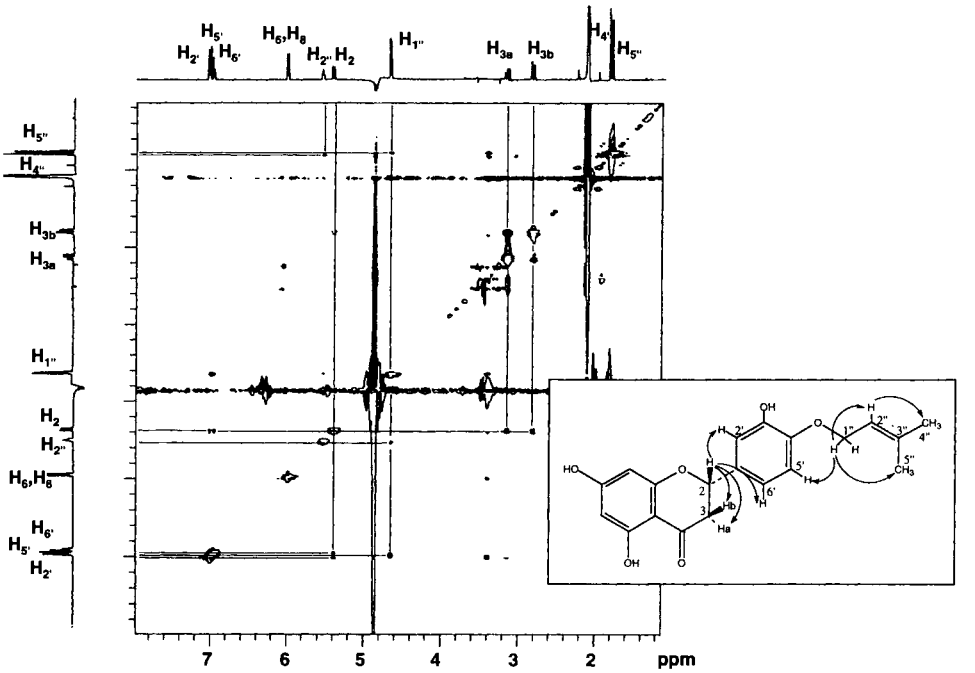


Fig. 4. *LC/WET-NOESY Spectrum of* **1** *in the active fraction of* M. engleri. Eluent system used: MeOH/D_2O (80:20); acquisition: 16 transients; 2 h 20 min time; 600 µg injected.

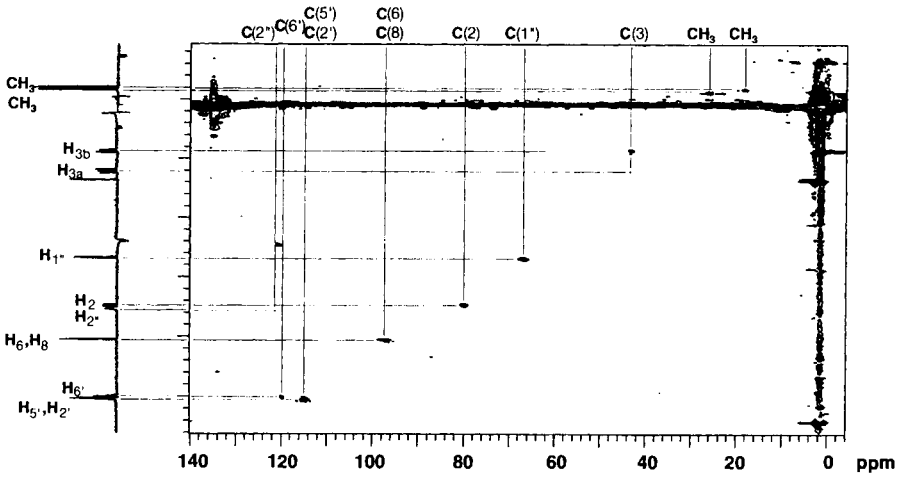


Fig. 5. *LC/GHSQC Spectrum of* **1** *in the active fraction of* M. engleri. Eluent system used: MeCN/D_2O (20:80 to 70:30 in 50 min). Acquisition: 4 transients; 128 increments; 29 min time; 1 mg of fraction injected.

Further purification of the fractions by gel filtration on *Sephadex LH-20* and semi-preparative HPLC afforded compounds **2**–**4**. As presumed from the LC/UV, LC/MS, and LC/NMR data, **2** was also an *O*-prenylated flavanone and was identified as *2,3-di-*

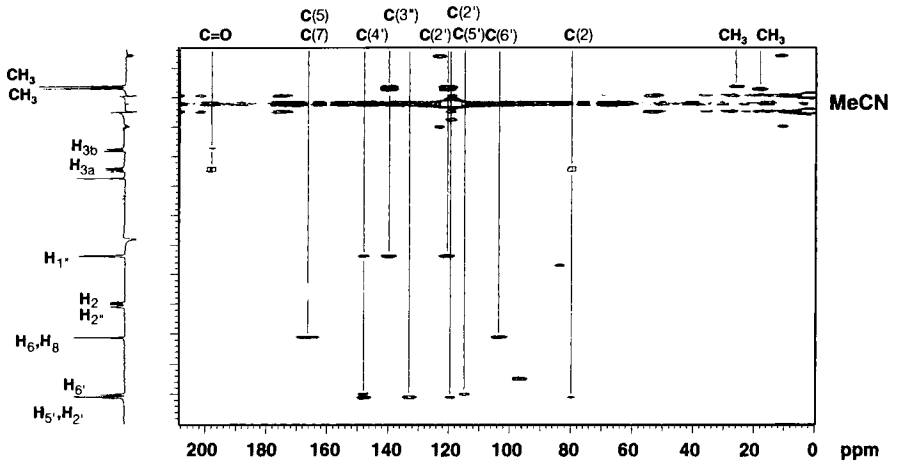


Fig. 6. *LC/GHMBC Spectrum of* **1** *in the active fraction of* M. engleri. Eluent system used: MeCN/D_2O (20:80 to 70:30 in 50 min). Acquisition: 96 transients; 6 h 20 min time; 1 mg of fraction injected.

*hydro-5,7-dihydroxy-2-{4-[(3-methylbut-2-enyl)oxy]phenyl}-4*H*-1-benzopyran-4-one*. This metabolite was isolate as a racemate. It is known as selinone, and has already been isolated from *Selinum vaginatum* (Apiaceae) [10].

Compounds **3** and **4** were flavanones with two prenyl units. The chains were meanwhile *C*-connected, as supported by the δ value of CH_2 at 3.18 ppm instead of 4.52 ppm for **2**. The EI mass spectra of the two compounds were also typical of *C*-prenylated products with fragment ions $[M - CH_3]^+$, $[M - C_3H_7]^+$, $[M - C_4H_7]^+$, and $[M - C_4H_7 - C_4H_8]^+$ [11]. The fragmentation of the *C*-prenylated flavanones by a *retro-Diels-Alder* (RDA) pathway is minor [11], but remains important for the structure elucidation. Compound **3** exhibited a $M^{+\cdot}$ peak at *m/z* 424, and the typical RDA fragment ion at *m/z* 136 ($[B_3]^+$) indicated the presence of two OH groups on the B-ring. In the ^{1}H-NMR, the substitution pattern of the aromatic protons on the B-ring was of the *AA'X* type, with two signals at 6.86 and 6.73 ppm, appearing as *singlets* in DMSO and exhibiting coupling in the COSY correlation experiment. When measured in $CDCl_3$, these two signals appeared as broad unresolved *multiplets*, confirming the coupling observed in the COSY. These data were in agreement with B-ring signals recorded for eriosemanone B, a *C*-prenylated flavanone, with a 3',5'-dihydroxy substitution [12]. These observations, in addition to GHMBC and 2D-NOESY spectra carried out on **3**, confirmed the position of the OH groups at C(3') and C(5'). Structure of **3**, monotesone B, was thus established as *2-(3,5-dihydroxyphenyl)-2,3-dihydro-5,7-dihydroxy-6,8-bis(3-methylbut-2-enyl)-4*H*-1-benzopyran-4-one*, a new natural product. In the same way, compound **4** was identified as lonchocarpol A, previously isolated from *Lonchocarpus minimiflorus* (Fabaceae) [13]. Both compounds were isolated as racemates.

The activity of compounds **1**–**4** against the microorganism *C. albicans* has been determined in a dilution assay using solid media [14]. In comparison with a blank, **2** inhibited growth of the yeast *Candida albicans* within 10 μg ml^{-1}, while **1** had a *MIC* of 20 μg ml^{-1} and **3** of 50 μg ml^{-1}. Compound **4** was devoid of any antifungal activity.

In the determination of *MIC* values, miconazole was used as positive control and inhibited the growth of the yeast within 0.1 μg ml^{-1}.

3. Discussion. – As shown here, the combined use of LC/UV/MS and LC/NMR provided numerous useful on-line data. The LC/UV/MS gave an idea on the type of structure of flavanones **1**–**4** and permitted to determine the molecular ions in the CH_2Cl_2 crude extract of *Monotes engleri*. LC/NMR On-flow allowed the detection of the different resonances of **1** and **2**, and confirmed the presence of prenylated flavanones in the extract. These data were, however, not sufficient for a complete identification but indicated interesting structural features for compound **1**. Even if a large quantity of the extract was injected (1 mg), it was not possible to detect flavanones **3** and **4** on-flow. Thus, the limitations of LC/NMR for this type of analysis are apparent.

To obtain more structural informations, it was necessary to enhance the sensitivity of detection, and a second LC/NMR analysis on an enriched fraction was performed in the stop-flow mode. This analysis allowed to record good-quality ^{1}H-NMR spectra, and a series of 2D-NMR experiments on the LC peak of **1** could be performed. These data were sufficient for a full structural elucidation of **1**. However, as compound **1** possesses a chirality center, its isolation was necessary, and the absolute configuration was determined by CD.

This example also demonstrated the importance of the LC solvent system used in LC/NMR. Indeed, the complete structure elucidation of **1** could be carried out in the MeOH/D_2O solvent system only. In the MeCN/D_2O system, the substitution pattern of B-ring was not fully determined. The choice of the eluent is not only important for the chromatography, but also for the resolution of the LC/NMR signals of interest. To quantify the antifungal activity of different flavanones and to confirm their structures, **2**–**4** were also isolated. The *MIC* value against *Candida albicans* was determined for all prenylated flavanones **1**–**4**. The *O*-prenylated compounds **1** and **2** were the most active ones with a *MIC* value of 20 μg ml^{-1} and 10 μg ml^{-1}, respectively, but this activity was rather weak in comparison with commercialized antifungal agents.

This study has shown the potential of LC/NMR as a complement to LC/UV/MS for an on-line identification of flavanones. It gives strategical on-line information prior to the fractionation of the crude extract and, thus, avoids the isolation of common natural products. However, if more detailed, structure informations are needed, analyses on enriched fractions have to be performed, because of the relative low sensibility of LC/NMR.

Financial support has been provided by the *Swiss National Science Foundation* and the *Herbette Foundation* of the Faculty of Sciences, University of Lausanne.

Experimental Part

General. M.p. *Mettler-FP-80/82* hot-stage apparatus, uncorrected. α_D: *Perkin-Elmer-241* polarimeter. $[\alpha]_D^{23}$ (solvent, *c* in g sample in 100 ml solvent). UV: *Varian DMS 100* spectrophotometer, recorded in MeOH; λ_{max} [nm] (log ε). CD: *Jobin-Yvon-Isa Dichrograph-6*, recorded in MeOH with cell length 0.20 cm; λ_{max} [nm] ($\Delta\varepsilon$). Medium-pressure liquid chromatography (MPLC): home-packed silica gel *60* (40–63 μm, 500 × 26 mm i.d.; *Merck*). Column chromatography (CC): *Sephadex LH-20* (*Pharmacia*). Anal. HPLC: *Hewlett-Packard-1090* instrument equipped with a photodiode array detector (DAD). Fractions were analyzed on *Nova-pak* C_{18} columns (4 μm, 150 mm × 3.9 mm i.d.; *Waters*) with a gradient of MeCN 20–70% in 50 min, at a flow rate of 1 ml min^{-1}, ^{1}H and

^{13}C-NMR: *Varian-Innova-500* spectrometer (499.870 and 125.704 MHz, resp.); recorded in DMSO and MeOH; δ in ppm rel. to Me_4Si, J in Hz. MS: *Finnigan-MAT-TSQ-700* triple stage quadrupole instrument; *m/z* (rel. intensity in %); EI-MS: ionization energy 70 eV; D/CI-MS: NH_3, positive-ion mode. Bioassays: dilution assays were carried out in *malt agar* media for *Candida albicans*. The pure compounds were assayed at 1, 10, 50, and 100 μg ml^{-1}.

LC/MS Analysis. For LC/TSP-MS analysis, an aq. buffer of 0.5M NH_4OAc was added post-column (0.2 ml/ml) to induce ionization (*Waters-590-MS* pump). TSP-MS: *Thermospray 2* (*Finnigan MAT*) interface, source temp. 280°, vaporizer 100°, aerosol 280°–360°, electron-multipler voltage 1800 V, dynode 15 kV, filament off and positive-ion mode, detection on a *Finnigan-MAT-TSQ-700* triple quadrupole instrument; spectra (150–900 amu) were recorded every 3 s.

LC/NMR Analyses. Varian-Innova-500 spectrometer equipped with a ^{1}H[^{13}C] pulse field gradient indirect microflow LC/NMR probe (flow cell 60 μl; 3 mm i.d.) was used. Reversed-phase HPLC of the crude extract and the active fraction was carried out on a *Varian* modular HPLC system, comprising a *Varian 9012* pump, a *Valco* injection valve, and a *Varian 9050* UV detector. The *Varian* HPLC software employed also enable programmable stop-flow experiments to be carried out.

Plant Material. Whole plants of *Monotes engleri* GILG were collected near Mutoko, Zimbabwe. A voucher specimen has been deposited at the National Herbarium of Zimbabwe, Causeway, Harare.

Extraction and Isolation. At r.t., 200 g of dry powdered leaves were successively extracted with CH_2Cl_2 and MeOH (3 × 2500 ml) to afford 5.7 g and 44.8 g of extract, respectively. The CH_2Cl_2 extract was fractionated by MPLC on SiO_2 into 13 fractions (*I–XIII*) with a step-gradient elution (petroleum ether/AcOEt, 10:1 → 1:2) and finally MeOH. Compound **1** (89 mg) was obtained from *Fr. X* by gel filtration on *Sephadex LH-20* ($CHCl_3$/MeOH 1:1). Fractionation of *Fr. VIII* by gel filtration on *Sephadex LH-20* ($CHCl_3$/MeOH 1:1) and further purification by semi-prep. HPLC on *Symmetry RP-18* (MeOH/H_2O 75:25) provided pure **2** (7 mg) and **3** (3 mg). Compound **4** (3 mg) was obtained from *Fr. XI* by gel filtration ($CHCl_3$/MeOH 1:1) and final purification by semi-prep. HPLC on *Symmetry RP-18* (MeOH/H_2O 75:25).

*(−)-(2S)-2,3-Dihydro-5,7-dihydroxy-2-{3-hydroxy-4-[(3-methylbut-2-enyl)oxy]phenyl}-4*H*-1-benzopyran-4-one* (= *Monotesone A*; **1**). M.p. 81–83°. $[\alpha]_D^{23} = -24.7$ ($c = 0.32$, MeOH). UV: 328 (3.33), 288 (4.15), 225 (sh, 4.27), 220 (4.29); ($AlCl_3$) 312; ($AlCl_3$ + HCl) 307; (NaOAc) 324; (NaOMe) 323. CD (MeOH, $c = 0.353$ mmol/l): 328 (2.14), 292 (−11.54), 251 (0.52), 220 (8.10). ^{1}H-NMR (MeOH): 6.94 (*d*, $J = 2.2$, H–C(2′)); 6.90 (*d*, $J = 8.4$, H–C(5′)); 6.85 (*dd*, $J = 2.2, 8.4$, H–C(6′)); 5.89 (*d*, $J = 2.5$, H–C(6)); 5.87 (*d*, $J = 2.5$, H–C(8)); 5.49 (*m*, $J = 6.8$, H–C(2″)); 5.28 (*dd*, $J = 2.9, 12.7$, H–C(2)); 4.58 (*d*, $J = 6.8$, H–C(1″)); 3.03 (*dd*, $J = 12.7, 17.1$, H_a–C(3)); 2.69 (*dd*, $J = 2.9, 17.1$, H_b–C(3)); 1.77 (*s*, Me); 1.73 (*s*, Me). ^{13}C-NMR (MeOH): 197.5 (C(4)); 168.4; 165.4; 164.7; 148.2; 148.1 (C(4′)); 138.6 (C(3″)); 133.2 (C(1′)); 121.3 (C(2″)); 118.9 (C(6′)); 114.6 (C(2′)); 114.4 (C(5′)); 103.3 (C(10)); 97.1 (C(8)); 96.2 (C(6)); 80.2 (C(2)); 66.9 (C(1″)); 44.0 (C(3)); 25.9 (C(4″)); 18.2 (C(5″)). EI-MS: 356 (27, M^+), 288 (100, $[M - C_5H_8]^+$), 179 (17), 166 (39), 153 (49, $[A_1 + H]^+$).

*(±)-2,3-Dihydro-5,7-dihydroxy-2-{4-[(3-methylbut-2-enyl)oxy]phenyl}-4*H*-1-benzopyran-4-one* (**2**). $[\alpha]_D^{23} = 0$ ($c = 0.29$, MeOH). UV: 289 (4.05), 209 (4.27). ^{1}H-NMR (DMSO): 7.39 (*d*, $J = 8.8$, H–C(2′), H–C(6′)); 6.94 (*d*, $J = 8.8$, H–C(3′), H–C(5′)); 5.71 (*d*, $J = 7.3$, H–C(6), H–C(8)); 5.41 (*m*, H–C(2), H–C(2″)); 4.52 (*d*, $J = 6.4$, H–C(1″)); 3.14 (*dd*, $J = 12.7, 16.6$, H_a–C(3)); 6.64 (*dd*, $J = 2.9, 16.6$, H_b–C(3)). EI-MS: 340 (4, M^+), 272 (100, $[M - C_5H_8]^+$), 153 (36, $[A_1]^+$).

*(±)-2-(3,5-Dihydroxyphenyl)-2,3-dihydro-5,7-dihydroxy-6,8-bis(3-methylbut-2-enyl)-4*H*-1-benzopyran-4-one* (= *Monotesone B*; **3**). $[\alpha]_D^{23} = 0$ ($c = 0.06$, $CHCl_3$). UV: 292 (4.75), 214 (4.99); ($AlCl_3$): 295; ($AlCl_3$ + HCl) 395; (NaOAc) 340; (NaOMe) 340. ^{1}H-NMR (DMSO): 12.44 (*s*, HO–C(5)); 8.96 (*s*); 6.86 (*s*, H–C(4′)); 6.73 (*s*, H–C(2′), H–C(6′)); 5.28 (*dd*, $J = 3.0, 12.2$, H–C(2)); 5.07 (*m*, H–C(2″)); 3.16 (*dd*, $J = 7.9, 11.7$, H–C(1″)); 3.06 (*dd*, $J = 12.2, 17.1$, H_a–C(3)); 2.66 (*dd*, $J = 3.0, 17.1$, H_b–C(3)); 1.70 (*s*, Me); 1.61 (*s*, Me); 1.59 (*s*, Me); 1.55 (*s*, Me). ^{1}H-NMR ($CDCl_3$): 12.31 (*s*, HO–C(5)); 6.98 (*m*, H–C(4′)); 6.88 (*m*, H–C(2′), H–C(5′)); 5.25 (*m*, H–C(2″), H–C(2)); 3.31 (*dd*, $J = 6.4$, 19.5, H–C(1″)); 3.00 (*dd*, $J = 6.2$, 16.6, H_a–C(3)); 2.79 (br. *d*, $J = 16.6$, H_b–C(3)); 1.81 (*s*, Me); 1.75 (*s*, Me); 1.72 (*s*, 2 Me). EI-MS: 424 (100, M^+), 409 (12, $[M - CH_3]^+$), 381 (10, $[M - C_3H_7]^+$), 369 (36, $[M - C_4H_7]^+$), 353 (40), 325 (15), 313 (20, $[M - C_4H_7 - C_4H_8]^+$), 273 (22), 245 (14), 233 (30, $[A_1 - 55]^+$), 217 (28), 189 (48), 177 (30, $[A_1 - C_4H_7 - C_4H_8]^+$), 136 (12, $[B_3]^+$), 123 (10).

*(±)-2,3-Dihydro-5,7-dihydroxy-2-(4-hydroxyphenyl)-6,8-bis(3-methylbut-2-enyl)-4*H*-1-benzopyran-4-one* (**4**). $[\alpha]_D^{23} = 0$ ($c = 0.14$, $CHCl_3$). UV: 295 (4.60), 214 (4.87). ^{1}H-NMR (DMSO): 12.37 (*s*, H–C(5)); 9.47 (*s*, H–C(4′)); 7.29 (*d*, $J = 8.9$, H–C(2′), H–C(6′)); 6.78 (*d*, $J = 8.7$, H–C(3′), H–C(5′)); 5.38 (*dd*, $J = 3.0, 12.7$, H–C(2)); 5.08 (*dd*, $J = 6.8, 14.2$, H–C(2″)); 3.22 (*m*, H–C(1″)); 3.20 (*m*, H_b–C(3)); 2.74 (*dd*, $J = 17.1, 3.0$, H_a–C(3)); 1.70 (*s*, Me); 1.62 (*s*, Me); 1.59 (*s*, Me); 1.53 (*s*, Me). EI-MS: 408 (100, M^+), 393 (18, $[M - CH_3]^+$),

365 (18, $[M - C_3H_7]^+$), 353 (38, $[M - C_4H_7]^+$), 337 (44), 297 (25, $[M - C_4H_7 - C_4H_8]^+$), 273 (28), 233 (30, $[A_1 - 55]^+$), 217 (35), 189 (80), 177 (40), 120 (60, $[B_3]^+$), 107 (35, $[B_4]^+$), 91 (28), 69 (16).

REFERENCES

[1] M. Gelfand, S. Mavi, R. B. Drummond, B. Ndemera, 'The Traditional Medical Practitioner in Zimbabwe', Mambo Press, Gweru, 1985.
[2] L. Rahalison, M. Hamburger, K. Hostettmann, M. Monod, E. Frenk, *Phytochem. Anal.* **1991**, *2*, 199.
[3] K. R. Markham, 'Techniques of Flavonoid Identification', Academic Press, London, 1982, pp. 36–52.
[4] E. K. Seo, G. L. Silva, H. B. Chai, T. E. Chagwedera, N. R. Farnsworth, G. A. Cordell, J. M. Pezzuto, A. D. Kinghorn, *Phytochemistry* **1997**, *45*, 509.
[5] A. L. Homans, A. Fuchs, *J. Chromatogr.* **1970**, *51*, 327.
[6] M. Kaouadji, P. Ravanel, A.-M. Mariotte, *J. Nat. Prod.* **1986**, *49*, 153.
[7] S. H. Smallcombe, S. L. Patt, P. A. Keiffer, *J. Magn. Reson.* **1995**, *117*, 295.
[8] W. Gaffield, *Tetrahedron* **1970**, *26*, 4093.
[9] G. Snatzke, F. Snatzke, A. L. Tökés, M. Rákosi, R. Bognár, *Tetrahedron* **1970**, *29*, 909.
[10] T. R. Seshadri, M. S. Sood, *Tetrahedron Lett.* **1967**, *9*, 853.
[11] A. V. Rama Rao, S. S. Rathi, K. Venkataraman, *Ind. J. Chem.* **1972**, *10*, 989.
[12] W. G. Ma, N. Fuzzati, Q. S. Li, C. R. Yang, H. Stoeckli-Evans, K. Hostettmann, *Phytochemistry* **1995**, *39*, 1049.
[13] V. Roussilis, S. A. Ampofo, D. F. Wiemer, *Phytochemistry* **1987**, *26*, 2317.
[14] L. Rahalison, Ph. D. Thesis, Lausanne, Switzerland, 1994.

Received December 29, 1997