# Tirucallane Triterpenoid Saponins from Munronia delavayi Franch 

by Xiang-Hai Cai $\left.{ }^{\mathrm{a}}\right)^{\mathrm{b}}$ ), Zhi-Zhi Du ${ }^{\text {a }}$ ), and Xiao-Dong Luo* ${ }^{*}$ )<br>${ }^{\text {a }}$ ) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, 320 Lanhei Road, Kunming 650204, P. R. China (phone: +86-871-5223177; fax: +86-871-5150227; e-mail: xdluo@mail.kib.ac.cn) ${ }^{\text {b }}$ ) Graduate School of Chinese Academy of Sciences, Beijing 100039, P. R. China


#### Abstract

Four new tirucallane triterpenoid saponins, named munronosides I-IV (2-5), along with three known triterpenoids, sapelin B (1), melianodiol, and (3 $\beta$ )-22,23-epoxytirucall-7-ene-3,24,25-triol, were isolated from the EtOH extract of the whole plants of Munronia delavayi Franch by chromatographic methods. On the basis of spectroscopic evidences, the structures of $\mathbf{2 - 5}$ were elucidated as ( $20 S, 23 R, 24 S$ )-21,25-epoxy-29-\{\{ $O$ - $\beta$-d-glucopyranosyl-( $1 \rightarrow 3$ )- $O$-[ $\alpha$-L-rhamnopyranosyl-( $1 \rightarrow 6$ )]- $\beta$-d-glucopyranosyl\}oxy\}-23,24-dihydroxytirucall-7-ene-3,21-dione (2), (3 $2,20 S, 23 R, 24 S)$-21,25-epoxy-29-$\{\{O-\beta$-D-glucopyranosyl- $(1 \rightarrow 3)$ - $O$-[ $\alpha$-L-rhamnopyranosyl-( $1 \rightarrow 6$ )]- $\beta$-d-glucopyranosyl $\}$ oxy $\}-3,23,24$-tri-hydroxytirucall-7-en-21-one (3), (20S,23R,24S)-24-(acetyloxy)-21,25-epoxy-29-\{\{O- $\beta$-d-glucopyranosyl$(1 \rightarrow 3)$ - $O$-[ $\alpha$-L-rhamnopyranosyl-( $1 \rightarrow 6$ )]- $\beta$-d-glucopyranosyl\}oxy\}-23-hydroxytirucall-7-ene-3,21-dione (4), and ( $3 \beta, 20 S, 23 R, 24 S$ )-24-(acetyloxy)-21,25-epoxy-29-\{\{ $O$ - $\beta$-D-glucopyranosyl-( $1 \rightarrow 3$ )- $O$ - $[\alpha$-L-rham-nopyranosyl-( $1 \rightarrow 6$ )]- $\beta$-D-glucopyranosyl\}oxy\}-3,23-dihydroxytirucall-7-en-21-one (5).


Introduction. - Plants of the family Meliaceae are a promising source of unique natural products for integrated pest management [1]. Plants of the genus Munronia are shrubs or half-shrubs which are naturally distributed in Sri Lanka, India, China, Indonesia, and from East Timor to the Philippines [2]. We previously studied Munronia henryi Harms collected from Xishuangbanna, in the south of Yunnan Province where the climate is warm and moist; many novel limonoids have been isolated, and some of them showed significant antifeedant properties [3]. Another species, Munronia delavayi Franch is narrowly distributed over the rocky area in the northeast of Yunnan Province. The markedly different plant habitats of the two species prompted us to investigate $M$. delavayi to see if the two plants differ in their secondary metabolites. Instead of limonoids as found in M. henryi, tirucallane triterpenoids, sapelin B (1) [4], melianodiol [5], (3 $\beta$ )-22,23-epoxytirucall-7-ene-3,24,25-triol [6], and four new tirucallane glycosides, munronosides I-IV $(\mathbf{2}-\mathbf{5})$ were isolated from the EtOH extract of M. delavayi Franch. This paper describes the isolation and structural determination of the new compounds.

Results and Discussion. - Compound 2 was found to possess the molecular formula $\mathrm{C}_{48} \mathrm{H}_{76} \mathrm{O}_{20}$ as evidenced by a quasimolecular-ion peak $[M-\mathrm{H}]^{-}$at $\mathrm{m} / \mathrm{z} 971.4836$ in the HR-ESI-MS. The ${ }^{1} \mathrm{H}-$ and ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (Tables 1 and 2) and DEPT spectra showed the presence of three sugar units, a rhamnose and two glucose units. The identification of the sugar residues was confirmed by acid hydrolysis of $\mathbf{2}$ with $10 \% \mathrm{HCl}$ solution. LRhamnose and D-glucose were identified in the hydrolysate by TLC comparison with

$1 \mathrm{R}^{1}=\alpha-\mathrm{OH}, \mathrm{R}^{2}=\mathrm{OH}, \mathrm{R}^{3}=\mathrm{H}, \mathrm{R}^{4}=\mathrm{H}, \mathrm{H}$
$3 \mathrm{R}^{1}=\beta-\mathrm{OH}, \mathbf{R}^{2}=\mathrm{OH}, \mathbf{R}^{3}=\mathbf{S}, \mathbf{R}^{4}=\mathrm{O}$
$5 \mathrm{R}^{1}=\beta-\mathrm{OH}, \mathrm{R}^{2}=\mathrm{AcO}, \mathrm{R}^{3}=\mathrm{S}, \mathrm{R}^{4}=\mathrm{O}$

$6 R^{1}=\mathrm{OH}, \mathrm{R}^{2}=\mathrm{H}, \mathrm{R}^{3}=\mathrm{H}, \mathrm{H}$
$2 \mathrm{R}^{1}=\mathrm{OH}, \mathrm{R}^{2}=\mathrm{S}, \mathrm{R}^{3}=\mathrm{O}$
$4 R^{1}=A c O, R^{2}=S, R^{3}=O$



Figure. Key HMBC Correlations of $\mathbf{2}$
authentic samples and determination of their specific optical rotation [7]. Comparison of the ${ }^{13} \mathrm{C}$-NMR and DEPT spectra of 2 with those of sapelin B (1) and hispidone (6) [8] and HMBC (Fig.), HSQC, ${ }^{1} \mathrm{H},{ }^{1} \mathrm{H}$-COSY, and ROESY data allowed to determine the structure of $\mathbf{2}$ as $(20 S, 23 R, 24 S)$-21,25-epoxy-29-\{\{ $O$ - $\beta$-D-glucopyranosyl- $(1 \rightarrow 3)$ - $O$ [ $\alpha$-L-rhamnopyranosyl-( $1 \rightarrow 6$ )]- $\beta$-D-glucopyranosyl\}oxy\}-23,24-dihydroxytirucall-7-ene3,21 -dione, which was named munronoside I.

The four ${ }^{1} \mathrm{H}-\mathrm{NMR}$ signals of 2 at $\delta(\mathrm{H}) 4.82(s, 1 \mathrm{H}), 4.65(d, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 4.37(d, J=7.5 \mathrm{~Hz}$, $1 \mathrm{H})$, and $1.34(d, J=6.0 \mathrm{~Hz}, 3 \mathrm{H})$, the ${ }^{13} \mathrm{C}$-NMR signals of three anomeric C-atoms $(\delta(\mathrm{C}) 104.5(d)$, $102.5(d)$, and $102.0(d)$ ), of a $\mathrm{Me}(\delta(\mathrm{C}) 18.3)$, two $\mathrm{CH}_{2}(\delta(\mathrm{C}) 68.0$ and 62.3$)$, and 12 CH groups $(\delta(\mathrm{C}) c a$. $69-82)$ were consistent with a rhamnose and two glucose units. The $s$ at $\delta(\mathrm{H}) 4.82$ indicated the $\alpha$ orientation of the anomeric proton of L-rhamnose. Likewise, the $J$ values of the other two anomeric protons of the sugar moieties revealed the $\beta$-configuration of the glucose residues.

Table 1. ${ }^{13} \mathrm{C}$-NMR Data of Compounds 2, 4, and $\mathbf{5}$ ( 100 MHz ), and of $\mathbf{3}$ ( 125 MHz )

|  | $2\left(\mathrm{CD}_{3} \mathrm{OD}\right)$ | $3\left(\mathrm{CD}_{3} \mathrm{OD}\right)$ | $4\left(\mathrm{C}_{5} \mathrm{D}_{5} \mathrm{~N}\right)$ | $5\left(\mathrm{CD}_{3} \mathrm{OD}\right)$ |
| :---: | :---: | :---: | :---: | :---: |
| C(1) | 40.7 ( $t$ ) | 38.6 (t) | 39.5 (t) | 38.6 (t) |
| C(2) | 37.1 (t) | 29.2 (t) | 36.7 ( $t$ ) | 29.2 (t) |
| C(3) | 217.7 (s) | 81.4 (d) | 214.9 (s) | 81.4 (d) |
| C(4) | 53.8 (s) | 42.9 (s) | 53.2 (s) | 42.9 (s) |
| C(5) | 55.8 (d) | 53.5 (d) | 55.0 (d) | 53.5 (d) |
| C(6) | 24.7 (t) | 24.7 (t) | 24.2 (t) | 24.7 (t) |
| C(7) | 119.3 ( $t$ ) | 119.2 (d) | 118.7 (d) | 119.2 (d) |
| C(8) | 146.9 (s) | 146.5 (s) | 145.9 (s) | 146.4 (s) |
| C(9) | 49.2 (d) | 50.4 (d) | 48.2 (d) | 48.6 (d) |
| C(10) | 36.5 (s) | 35.9 (s) | 35.6 (s) | 35.9 (s) |
| C(11) | 18.8 ( $t$ ) | 18.8 ( $t$ ) | 18.8 ( $t$ ) | 18.8 ( $t$ ) |
| $\mathrm{C}(12)$ | 32.1 (t) | 32.2 (t) | 31.5 ( $t$ ) | $31.9(t)$ |
| C(13) | 44.7 ( $s$ ) | 44.8 ( $s$ ) | 44.1 ( $s$ ) | 44.8 ( $s$ ) |
| $\mathrm{C}(14)$ | 51.5 (s) | 51.6 (s) | 50.8 ( $s$ ) | 51.5 (s) |
| C(15) | 34.9 ( $t$ ) | 34.9 ( $t$ ) | 34.3 ( $t$ ) | 34.8 ( $t$ ) |
| C(16) | 26.0 (t) | 25.3 (t) | 25.5 ( $t$ ) | 25.3 ( $t$ ) |
| C(17) | 48.4 (d) | 48.4 (d) | 47.7 (d) | 48.4 (d) |
| C(18) | 23.9 (q) | 23.9 (q) | 23.5 (q) | 23.8 (q) |
| C(19) | 14.2 (q) | 14.5 (q) | 13.8 (q) | 14.5 (q) |
| $\mathrm{C}(20)$ | 41.5 (d) | 41.6 (d) | 41.1 (d) | 41.4 (d) |
| C(21) | 181.2 (s) | 181.3 (s) | 178.4 (s) | 180.5 (s) |
| $\mathrm{C}(22)$ | 31.6 (t) | 31.6 (t) | 32.1 (t) | 32.2 (t) |
| C(23) | 76.4 (d) | 76.7 (d) | 76.6 (d) | 76.6 (d) |
| $\mathrm{C}(24)$ | 78.8 (d) | 79.0 (d) | 79.5 (d) | 79.6 (d) |
| C(25) | 73.4 (s) | 73.4 (s) | 71.3 (s) | 72.1 (s) |
| C(26) | 25.0 (q) | 25.0 (q) | 25.9 (q) | 25.5 (q) |
| C(27) | 27.8 (q) | 27.8 (q) | 28.4 (q) | 27.5 (q) |
| C(28) | 20.7 (q) | 23.1 (q) | 20.8 (q) | 23.1 (q) |
| C(29) | 73.5 ( $t$ ) | 71.9 (t) | 72.8 (t) | 71.9 (t) |
| C(30) | 28.0 (q) | 27.8 (q) | 27.6 (q) | 27.8 (q) |
| AcO |  |  | 171.0 (s), 21.0 (q) | 172.3 (s), 20.9 (q) |
| C(1) | 102.0 (d) | 101.9 (d) | 102.6 (d) | 101.9 (d) |
| C(2') | 77.6 (d) | 77.8 (d) | 76.9 (d) | 77.4 (d) |
| $\mathrm{C}\left(3^{\prime}\right)$ | 81.4 (d) | 82.8 (d) | 82.2 (d) | 82.8 (d) |
| $\mathrm{C}\left(4^{\prime}\right)$ | 71.1 (d) | 71.0 (d) | 71.3 (d) | 71.0 (d) |
| C( $5^{\prime}$ ) | 78.1 (d) | 78.2 (d) | 78.5 (d) | 78.0 (d) |
| $\mathrm{C}\left(6^{\prime}\right)$ | 68.0 ( $t$ ) | 67.4 ( $t$ ) | 68.4 ( $t$ ) | 67.4 ( $t$ ) |
| $\mathrm{C}\left(1^{\prime \prime}\right)$ | 104.5 (d) | 105.3 (d) | 105.7 (d) | 105.3 (d) |
| $\mathrm{C}\left(2^{\prime \prime}\right)$ | 78.4 (d) | 78.5 (d) | 78.7 (d) | 78.2 (d) |
| $\mathrm{C}\left(3^{\prime \prime}\right)$ | 75.9 (d) | 75.7 (d) | 76.6 (d) | 75.6 (d) |
| $\mathrm{C}\left(4^{\prime \prime}\right)$ | 77.9 (d) | 78.0 (d) | 78.3 (d) | 77.8 (d) |
| $\mathrm{C}\left(5^{\prime \prime}\right)$ | 72.2 (d) | 72.3 (d) | 72.8 (d) | 72.4 (d) |
| $\mathrm{C}\left(6^{\prime \prime}\right)$ | 62.3 (t) | 61.9 (t) | 62.6 (t) | 61.9 (t) |
| $\mathrm{C}\left(1^{\prime \prime \prime}\right)$ | 102.5 (d) | 102.0 (d) | 102.7 (d) | 102.0 (d) |
| $\mathrm{C}\left(2^{\prime \prime \prime}\right)$ | 73.8 (d) | 73.9 (d) | 74.0 (d) | 73.9 (d) |
| $\mathrm{C}\left(3^{\prime \prime \prime}\right)$ | 72.0 (d) | 72.1 (d) | 72.4 (d) | 72.3 (d) |
| $\mathrm{C}\left(4^{\prime \prime \prime}\right)$ | 70.9 (d) | 70.3 (d) | 71.3 (d) | 70.3 (d) |
| $\mathrm{C}\left(5^{\prime \prime \prime}\right)$ | 69.7 (d) | 69.7 (d) | 69.9 (d) | 69.7 (d) |
| $\mathrm{C}\left(6^{\prime \prime \prime}\right)$ | 18.3 (q) | 18.2 (q) | 18.8 (q) | 18.2 (q) |

Table 2. ${ }^{1} H$-NMR Data ( 500 MHz ) of Compounds 2-5. $\delta$ in ppm, $J$ in Hz.

|  | $2\left(\mathrm{CD}_{3} \mathrm{OD}\right)$ | $3\left(\mathrm{CD}_{3} \mathrm{OD}\right)$ | $4\left(\mathrm{C}_{5} \mathrm{D}_{5} \mathrm{~N}\right)$ | $5\left(\mathrm{CD}_{3} \mathrm{OD}\right)$ |
| :---: | :---: | :---: | :---: | :---: |
| $\mathrm{CH}_{2}(1)$ | 1.50-1.53 (m), | 1.25-1.30 (m), | 1.25-1.29 (m), | 1.20-1.24 (m), |
|  | 2.14-2.18 (m) | 1.71-1.75 (m) | 1.81-1.85 (m) | 1.73-1.77 (m) |
| $\mathrm{CH}_{2}(2)$ | 2.29-2.31 (m), | 1.72-1.77 (m) | 1.96-2.00 (m), | 1.75-1.79 (m) |
|  | 2.99-3.02 (m) |  | 3.22-3.26 (m) |  |
| $\mathrm{H}-\mathrm{C}(3)$ | - | 3.23 ( $d d, J=8.0,4.5$ ) | - | 3.23 (dd, $J=8.0,4.0)$ |
| $\mathrm{H}-\mathrm{C}(5)$ | 1.76-1.80 (m) | 1.45 ( $d d, J=2.5,7.0$ ) | 1.76-1.80 (m) | 1.47-1.52 (m) |
| $\mathrm{CH}_{2}(6)$ | 1.60-1.64 (m), | $1.54-1.57$ (m), | 1.44-1.50 (m), | 1.45-1.51 (m), |
|  | 1.81-1.84 (m) | 1.77-1.80 (m) | 1.76-1.81 (m) | 1.77-1.82 (m) |
| $\mathrm{H}-\mathrm{C}(7)$ | 5.38 (br. s) | 5.27 (br. s) | 5.22 (br. s) | 5.28 (br.s) |
| $\mathrm{H}-\mathrm{C}(9)$ | 2.42-2.46 (m) | 2.25-2.29 (m) | 2.26-2.30 (m) | 2.27-2.32 (m) |
| $\mathrm{CH}_{2}(11)$ | 1.70-1.73 (m) , | 1.60-1.55 (m) | 1.44-1.49 (m) | 1.65-1.58 (m) |
|  | 1.78-1.81 (m) |  |  |  |
| $\mathrm{CH}_{2}(12)$ | 1.78-1.82 (m) | 1.74-1.82 (m) | 1.80-1.84 (m) | 1.73-1.79 (m) |
| $\mathrm{CH}_{2}(15)$ | 1.63-1.66 (m) | 1.53-1.58 (m) | 1.45-1.56 (m) | 1.55-1.61 (m) |
| $\mathrm{CH}_{2}(16)$ | 2.14-2.18 (m), | 1.90-1.95 (m), | 2.12-2.16 (m), | 1.95-2.01 (m), |
|  | 2.28-2.31 (m) | 2.23-2.29 (m) | 2.22-2.28 (m) | 2.22-2.27 (m) |
| $\mathrm{H}-\mathrm{C}(17)$ | 2.40-2.43 (m) | 2.30-2.35 (m) | 1.91-1.95 (m) | 2.29-2.33 (m) |
| $\mathrm{Me}(18)$ | 0.92 (s) | 0.86 (s) | 0.87 (s) | 0.84 (s) |
| $\mathrm{Me}(19)$ | 1.19 (s) | 0.76 (s) | 1.02 (s) | 0.77 (s) |
| $\mathrm{H}-\mathrm{C}(20)$ | 2.84-2.88 (m) | 2.79-2.82 (m) | 2.80-2.85 (m) | 2.79-2.82 (m) |
| $\mathrm{CH}_{2}$ (22) | 2.33-2.35 (m), | 2.18-2.22 (m) , | $2.22-2.26$ (m) , | 1.83-1.89 (m) , |
|  | 2.26-2.30 (m) | 2.26-2.30 (m) | 2.49-2.54 (m) | 2.38-2.43 (m) |
| $\mathrm{H}-\mathrm{C}(23)$ | 3.43-3.47 (m) | 3.40-3.45 (m) | 5.09-5.14 (m) | 3.38-3.42 (m) |
| $\mathrm{H}-\mathrm{C}(24)$ | 3.37 (br.s) | 3.27 (br. s) | $5.40(d, J=5.0)$ | $4.80(d, J=5.0)$ |
| $\mathrm{Me}(26)$ | 1.29 (s) | 1.23 (s) | 1.56 (s) | 1.27 (s) |
| Me (27) | 1.34 (s) | 1.34 (s) | 1.51 (s) | 1.22 (s) |
| Me (28) | 1.21 (s) | 1.21 (s) | 1.39 (s) | 1.22 (s) |
| $\mathrm{CH}_{2}$ (29) | $3.80(d, J=10.0)$, | $3.98(d, J=10.0)$, | $4.42(d, J=10.0)$, | 3.99 (d, $J=10.0)$, |
|  | 4.27 ( $d, J=10.0$ ) | $4.03(d, J=10.0)$ | 4.45 ( $d, J=10.0)$ | $4.02(d, J=10.0)$ |
| Me (30) | 1.14 (s) | 1.04 (s) | 0.96 (s) | 1.04 (s) |
| AcO | - | - | 2.12 (s) | 2.12 (s) |
| $\mathrm{H}-\mathrm{C}\left(1^{\prime}\right)$ | $4.37(d, J=7.5)$ | $4.38(d, J=8.0)$ | $4.79(d, J=8.0)$ | $4.39(d, J=7.5)$ |
| $\mathrm{H}-\mathrm{C}\left(2^{\prime}\right)$ | 3.42-3.45 (m) | 3.38-3.42 (m) | 3.93-3.95 (m) | 3.73-3.77 (m) |
| $\mathrm{H}-\mathrm{C}\left(3^{\prime}\right)$ | 3.23-3.27 (m) | 3.39-3.43 (m) | 4.12-4.16 (m) | 3.35-3.79 (m) |
| $\mathrm{H}-\mathrm{C}\left(4^{\prime}\right)$ | 3.37-3.41 (m) | 3.39-3.42 (m) | 3.89-3.92 (m) | 3.38-3.42 (m) |
| $\mathrm{H}-\mathrm{C}\left(5^{\prime}\right)$ | 3.62-3.66 (m) | $3.59(t, J=7.5)$ | 4.23-4.26 (m) | 3.19-3.24 (m) |
| $\mathrm{CH}_{2}\left(6^{\prime}\right)$ | $4.01-4.05(m)$ | $3.94-4.00(\mathrm{~m}),$ | $4.12-4.16(m)$ | $3.93-3.98(m)$ |
|  | $3.68-3.72(\mathrm{~m})$ | $3.68-3.73(m)$ | $4.55-4.59(\mathrm{~m})$ | $3.70-3.75(m)$ |
| $\mathrm{H}-\mathrm{C}\left(1^{\prime \prime}\right)$ | $4.65(d, J=8.0)$ | $4.62(d, J=7.5)$ | $5.35(d, J=7.5)$ | $4.62(d, J=8.0)$ |
| $\mathrm{H}-\mathrm{C}\left(2^{\prime \prime}\right)$ | 3.68-3.72 (m) | 3.68-3.72 (m) | 3.91-3.95 (m) | 3.58-3.62 (m) |
| $\mathrm{H}-\mathrm{C}\left(3^{\prime \prime}\right)$ | 3.35-3.38 (m) | 3.22-3.27 (m) | 4.10-4.15 (m) | 3.20-3.23 (m) |
| $\mathrm{H}-\mathrm{C}\left(4^{\prime \prime}\right)$ | 3.30-3.34 (m) | 3.20-3.26 (m) | 4.23-4.27 (m) | 3.35-3.40 (m) |
| $\mathrm{H}-\mathrm{C}\left(5^{\prime \prime}\right)$ | 3.74-3.78 (m) | 3.66-3.70 (m) | 4.22-4.26 (m) | 3.68-3.73 (m) |
| $\mathrm{CH}_{2}\left(6^{\prime \prime}\right)$ | $\begin{aligned} & 3.91-3.95(\mathrm{~m}) \\ & 3.81-3.85(\mathrm{~m}) \end{aligned}$ | $3.78-3.82(m)$ | $4.55-4.59(m)$ | $3.78-3.83(m)$ |
| $\mathrm{H}-\mathrm{C}\left(1^{\prime \prime \prime}\right)$ | 4.82 (s) | 4.75 (s) | 5.45 (s) | 4.76 (s) |
| $\mathrm{H}-\mathrm{C}\left(2^{\prime \prime \prime}\right)$ | 3.41-3.45 (m) | 3.40-3.43 (m) | 4.23-4.27 (m) | 3.35-3.39 (m) |
| $\mathrm{H}-\mathrm{C}\left(3^{\prime \prime \prime}\right)$ | 3.73-3.77 (m) | 3.81-3.85 (m) | 4.51-4.56 (m) | 3.80-3.85 (m) |
| $\mathrm{H}-\mathrm{C}\left(4^{\prime \prime \prime}\right)$ | 3.41-3.45 (m) | 3.41-3.45 (m) | 4.25-4.29 (m) | 3.38-3.42 (m) |
| $\mathrm{H}-\mathrm{C}\left(5^{\prime \prime \prime}\right)$ | 3.73-3.77 (m) | 3.68-3.72 (m) | 4.33-4.38 (m) | 3.68-3.73 (m) |
| $\mathrm{Me}\left(6^{\prime \prime \prime}\right)$ | $1.34(d, J=6.0)$ | $1.28(d, J=6.0)$ | $1.62(d, J=6.0)$ | $1.29(d, J=6.0)$ |

Besides of three sugar units, the ${ }^{13} \mathrm{C}$-NMR and DEPT spectra of 2 also showed 30 C -atoms, six Me , nine $\mathrm{CH}_{2}$, and seven CH groups, and eight quaternary C -atoms. These data were similar to those of hispidone (6), except for an additional $\mathrm{C}=\mathrm{O}$ at $\delta(\mathrm{C}) 181.2(s)$ and the loss of a Me group in $\mathbf{2}$. This $\mathrm{C}=\mathrm{O}$ group was located at $\mathrm{C}(21)$ by correlations between its $\delta(\mathrm{C}) 181.2(s)$ and $\delta(\mathrm{H}) 2.26-2.35\left(2 m, \mathrm{CH}_{2}(22)\right)$ and $2.84-2.88(m, H-C(20))$ in the HMBC spectrum (Fig.). Signals at $\delta(\mathrm{H}) 3.80$ and 4.27 (each $d, J=$ 10.0 Hz , each 1 H ) corresponding to $\delta(\mathrm{C}) 73.5(t)$ in its HSQC plot showed cross-peaks with $\delta(\mathrm{C}) 217.7(s$, $\mathrm{C}(3)), 102.0\left(d, \mathrm{C}\left(1^{\prime}\right)\right), 55.8(d, \mathrm{C}(5))$, and $20.7(q, \mathrm{C}(28))$, which suggested that O-substitution took place at $\mathrm{C}(29)(\delta(\mathrm{C}) 73.5(t))$. A ROESY correlation $\delta(\mathrm{H}) 1.76-1.80(m, \mathrm{H}-\mathrm{C}(5)) / \delta(\mathrm{H}) 1.21(s$, $\mathrm{Me}(28)$ ) confirmed the $\mathrm{CH}_{2}(29)-\mathrm{O}$ group. On the basis of the NOE correlations $\delta(\mathrm{H}) 3.43-3.47$ ( $m$, $\mathrm{H}-\mathrm{C}(23)) / \delta(\mathrm{H}) 1.34(s, \mathrm{Me}(27))$ and $\delta(\mathrm{H}) 3.37$ (br. $s, \mathrm{H}-\mathrm{C}(24)) / \delta(\mathrm{H}) 1.29(s, \mathrm{Me}(26))$, the configuration of $\mathrm{OH}-\mathrm{C}(23)$ and $\mathrm{OH}-\mathrm{C}(24)$ were determined to be trans. The glycosylation position was unambiguously determined to be at $\mathrm{C}(29)$ from the HMBC cross-peak $\delta(\mathrm{H}) 4.37(d, J=7.5 \mathrm{~Hz}$, $\left.\mathrm{H}-\mathrm{C}\left(1^{\prime}\right)\right) / \delta(\mathrm{C}) 73.5(\mathrm{C}(29)) . \mathrm{HMBC}$ Cross-peaks $\delta(\mathrm{H}) 4.37\left(\mathrm{H}-\mathrm{C}\left(1^{\prime}\right)\right) / \delta(\mathrm{C}) 81.4(d)$ and $78.1(d)$ showed the two latter signals arising from $\mathrm{C}\left(3^{\prime}\right)$ and $\mathrm{C}\left(5^{\prime}\right)$, respectively. ${ }^{1} \mathrm{H},{ }^{1} \mathrm{H}$-COSY Cross-peaks $\delta(\mathrm{H})$ $3.62-3.66\left(m, \mathrm{H}-\mathrm{C}\left(5^{\prime}\right)\right) / \delta(\mathrm{H}) 4.01-4.05$ and $3.68-3.72\left(2 m, \mathrm{CH}_{2}\left(6^{\prime}\right)\right)$, and the HSQC data enabled to assign $\delta(\mathrm{C}) 68.0(t)$ to $\mathrm{C}\left(6^{\prime}\right)$. On the basis of the correlations $\delta(\mathrm{H}) 4.65\left(d, J=8.0 \mathrm{~Hz}, \mathrm{H}-\mathrm{C}\left(1^{\prime \prime}\right)\right), \delta(\mathrm{C})$ $81.4\left(d, \mathrm{C}\left(3^{\prime}\right)\right)$ and $\delta(\mathrm{H}) 4.82\left(s, \mathrm{H}-\mathrm{C}\left(1^{\prime \prime \prime}\right)\right), \delta(\mathrm{C}) 68.0\left(t, \mathrm{C}\left(6^{\prime}\right)\right)$, the presence of a glucopyranosyl- $(1 \rightarrow$ 3)-[rhamnopyranosyl-( $1 \rightarrow 6$ )]-glucopyranosyl moiety was deduced.

Compound 3 has the molecular formula $\mathrm{C}_{48} \mathrm{H}_{78} \mathrm{O}_{20}$ as deduced from the quasimolecular-ion peak $[M-\mathrm{H}]^{-}$at $m / z 973.5021$ in the HR-ESI-MS. The structure of 3 was deduced to be $(3 \beta, 20 S, 23 R, 24 S)$-21,25-epoxy-29-\{\{ $O$ - $\beta$-d-glucopyranosyl$(1 \rightarrow 3)$ - $O$-[ $\alpha$-L-rhamnopyranosyl- $(1 \rightarrow 6)]$ - $\beta$-D-glucopyranosyl $\}$ oxy $\}-3,23,24$-trihydrox-ytirucall-7-en-21-one, by comparison with the data of $\mathbf{2}$, and named munronoside II.

The ${ }^{13} \mathrm{C}$-NMR (Table 1) and DEPT spectra of $\mathbf{3}$ displayed similarities to those of 2, except for the absence of the signal at $\delta(\mathrm{C}) 217.7(s, \mathrm{C}(3))$ in $\mathbf{3}$ which was replaced by $\delta(\mathrm{C}) 81.4(d, \mathrm{C}(3))$, indicating an OH substitution at $\mathrm{C}(3)$. This assumption was supported by the HMBC cross-peaks $\delta(\mathrm{H}) 3.23(d d, J=$ $8.0,4.5 \mathrm{~Hz}, \mathrm{H}-\mathrm{C}(3)) / \delta(\mathrm{C}) 23.1(q, \mathrm{C}(28)), 42.9(s, \mathrm{C}(4))$, and $53.5(d, \mathrm{C}(5))$. The large coupling constant of $\mathrm{H}-\mathrm{C}(3)($ Table 2$)$ indicated the $\beta$-orientation of the OH group [9].

Compound 4 possesses the molecular formula $\mathrm{C}_{50} \mathrm{H}_{78} \mathrm{O}_{20}$ as shown by the quasimolecular-ion peak $[M-\mathrm{H}]^{-}$at $\mathrm{m} / \mathrm{z} 1013.4946$ in the HR-ESI-MS. The structure of 4 was deduced to be ( $20 S, 23 R, 24 S$ )-24-(acetyloxy)-21,25-epoxy-29-\{\{O- $\beta$-d-gluco-pyranosyl-( $1 \rightarrow 3$ )-O-[ $\alpha$-L-rhamnopyranosyl-( $1 \rightarrow 6$ )]- $\beta$-d-glucopyranosyl $\}$ oxy $\}-23$-hy-droxytirucall-7-ene-3,21-dione and named munronoside III.

The ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}$-NMR (Tables 1 and 2) and DEPT spectra of $\mathbf{4}$ displayed similarities to those of 2, except for additional signals of an AcO group $(\delta(\mathrm{C}) 171.0(s)$ and $21.0(q))$. Location of the AcO group could be assigned to $\mathrm{C}(24)$ by the HMBC cross-peak $\delta(\mathrm{C}) 171.0(s) / \delta(\mathrm{H}) 5.40(d, J=5.0 \mathrm{~Hz}, \mathrm{H}-\mathrm{C}(24))$.

Compound 5 was found to possess the molecular formula $\mathrm{C}_{50} \mathrm{H}_{80} \mathrm{O}_{21}$ as evidenced by a quasimolecular ion peak $[M-H]^{-}$at $m / z 1015.5097$ in the HR-ESI-MS. The structure of 5 was deduced to be $(3 \beta, 20 S, 23 R, 24 S)$-24-(acetyloxy)-21,25-epoxy-29-\{\{O- $\beta$-d-glucopyranosyl- $(1 \rightarrow 3)$ - $O$-[ $\alpha$-L-rhamnopyranosyl- $(1 \rightarrow 6)]-\beta$-D-glucopyranosyl $\}$ oxy $\}$ -3,23-dihydroxytirucall-7-en-21-one and named munronoside IV.

The ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}$-NMR (Tables 1 and 2) and DEPT spectra of 5 showed similarities to those of $\mathbf{3}$, except for an additional AcO group $(\delta(\mathrm{C}) 172.3(s)$ and $20.9(q))$. The AcO group was located at $\mathrm{C}(24)$, as in 4 as established by from the HMBC cross-peak $\delta(\mathrm{C}) 172.3(s) / \delta(\mathrm{H}) 4.80(d, J=5.0 \mathrm{~Hz}, \mathrm{H}-\mathrm{C}(24)$.

Compounds $2-5$ all possess a new aglycone differing from previously reported compounds, in particular in the presence of an oxo substituent at $C(21)$. The configuration of $\mathrm{OH}-\mathrm{C}(3)$ in $\mathbf{3}$ and $\mathbf{5}$ is $\beta$ instead of $\alpha$, and an AcO group replaces the OH group at $\mathrm{C}(24)$ in the case of $\mathbf{4}$ and $\mathbf{5}$.

The authors are grateful to the National Natural Science Foundation of China (project No. 30370160, C30670214), the Natural Science Foundation of Yunnan Province (2004C0009Z), and the Chinese Academy of Science (XiBuZhiGuang project) for the financial support, and to the members of the analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for the spectral measurements.

## Experimental Part

General. Column chromatography (CC): Silica gel (200-300 mesh; Qindao Marine Chemical Factory, Qindao, China). TLC: Silica gel $G F_{254}$ (Qindao Marine Chemical Factory); detection by spraying with $5 \% \mathrm{H}_{2} \mathrm{SO}_{4}$ soln. and heating of the plate. Optical rotations: Horiba SEAP-300 spectropolarimeter IR Spetra: $\tilde{v}$ in $\mathrm{cm}^{-1}$. 1D- and 2D-NMR Spectra: Bruker AM-400 and DRX-500 NMR spectrometer; $\mathrm{SiMe}_{4}$ as internal standard, $\delta$ in ppm, $J$ in Hz. MS: VG Autospec-3000 and Finnigan MAT-90 spectrometers for FAB, and API Qstar-Pulsar-1 spectrometer for HR-ESI; in $m / z$ (rel.\%).

Plant Material. The whole plant of M. delavayi Franch was collected in QianJia County, Yunnan Province, People's Republic of China, in May 2004, and identified by Mr. Li-Shan Xie, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No.0848852) was deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried whole plant ( 11.5 kg ) was crushed and extracted with $90 \% \mathrm{aq}$. $\mathrm{EtOH}(4 \times 40 \mathrm{l})$ at r.t. $(4 \times 48 \mathrm{~h})$. After evaporation of the EtOH , the viscous residue was partitioned with $\mathrm{AcOEt}(4 \times 15 \mathrm{l})$ to afford an AcOEt and an $\mathrm{H}_{2} \mathrm{O}$ extract. The AcOEt extract $(210 \mathrm{~g})$ was subjected to CC (silica gel ( 2.0 kg ), $\mathrm{CHCl}_{3} / \mathrm{Me}_{2} \mathrm{CO} 1: 0 \rightarrow 1: 1$ ): Fractions I-VIII. Fr. VI ( 21.0 g ) was subjected to CC (silica gel ( 600 g ), petroleum ether $/ \mathrm{Me}_{2} \mathrm{CO} 3: 1$ ): $\mathbf{1}(21 \mathrm{mg})$ and melianodiol ( 28 mg ). Fr. VII $(15.5 \mathrm{~g})$ was subjected to CC (silica gel, $\mathrm{CHCl}_{3} / \mathrm{Me}_{2} \mathrm{CO} 8: 2$ ): (3 3 )-22,23-Epoxytirucall-7-ene-3,24,25-triol ( 25 mg ). The $\mathrm{H}_{2} \mathrm{O}$ extract (see above) was partitioned with butan- 1 -ol. After evaporation of the butan-1-ol, the residue ( 280 g ) was subjected to CC (silica gel ( 3.0 kg ), $\mathrm{CHCl}_{3} / \mathrm{MeOH} 9: 1 \rightarrow 2: 1$ ): Fr. $I X-$ XIII. Fr. XI $(14 \mathrm{~g})$ was subjected to $\mathrm{CC}\left(R P-18(300 \mathrm{~g}), \mathrm{MeOH} / \mathrm{H}_{2} \mathrm{O} 4: 6 \rightarrow 6: 4\right): \mathbf{2}(75 \mathrm{mg}), \mathbf{3}(56 \mathrm{mg}), \mathbf{4}(32 \mathrm{mg})$, and 5 ( 65 mg ).
(20S,23R,24S)-21,25-Epoxy-29-f\{O- $\beta$-D-glucopyranosyl-( $1 \rightarrow 3$ )-O-[ $\alpha$-L-rhamnopyranosyl-( $1 \rightarrow 6$ )]-$\beta$-D-glucopyranosylJoxy)-23,24-dihydroxytirucall-7-ene-3,21-dione ( $=(4 \beta, 13 \alpha, 14 \beta, 17 \alpha, 20 \mathrm{~S}, 23 \mathrm{R}, 24 \mathrm{~S})-29$ -[/O-6-Deoxy- $\alpha$-L-mannopyranosyl-( $1 \rightarrow 6$ )-O-[ $\beta$-D-glucopyranosyl-( $1 \rightarrow 3$ )]- $\beta$-D-glucopyranosyl]oxy]-21,25-epoxy-23,24-dihydroxylanost-7-ene-3,21-dione; 2): White powder. $[\alpha]_{\mathrm{D}}^{22}=-68.7(c=0.12, \mathrm{MeOH})$. IR (KBr): 3424, 2935, 1762, 1703, 1637. ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}$-NMR: Tables 1 and 2. FAB-MS (glycerol; neg.): 971 ( $100,[\mathrm{M}-\mathrm{H}]^{-}$). HR-ESI-MS: $971.4836\left(\mathrm{C}_{48} \mathrm{H}_{75} \mathrm{O}_{20}^{-}\right.$; calc. 971.4851).
(3及,20S,23R,24S)-21,25-Epoxy-29-\{fO- $\beta$-d-glucopyranosyl-( $1 \rightarrow 3$ )-O-[ $\alpha$-L-rhamnopyranosyl-( $1 \rightarrow$ 6)]- $\beta$-D-glucopyranosylfoxy $\}$-3,23,24-trihydroxytirucall-7-en-21-one $(=(3 \beta, 4 \beta, 13 \alpha, 14 \beta, 17 \alpha, 20 \mathrm{~S}, 23 \mathrm{R}$, $24 \mathrm{~S})$-29-\{/O- 6 -Deoxy- $\alpha$-L-mannopyranosyl-( $(1 \rightarrow 6)$-O-[ $\beta$-D-glucopyranosyl-( $(\rightarrow 3)]$ - $\beta$-D-glucopyrano-syljoxy]-21,25-epoxy-3,23,24-trihydroxylanost-7-en-21-one; 3): White powder. $[\alpha]_{D}^{22}=-57.6$ ( $c=0.17$, MeOH). IR (KBr): 3423, 2932, 1760, 1740. ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}$-NMR: Tables 1 and 2. FAB-MS (glycerol; neg.): 973 ( $100,[M-H]^{-}$). HR-ESI-MS: $973.5021\left(\mathrm{C}_{48} \mathrm{H}_{77} \mathrm{O}_{20}^{+}\right.$, calc. 973.5008$)$.
(20S,23R,24S)-24-(Acetyloxy)-21,25-epoxy-29-f/O- $\beta$-D-glucopyranosyl-( $1 \rightarrow 3$ )-O-[ $\alpha$-L-rhamnopyr-anosyl-( $1 \rightarrow 6$ ) ]- $\beta$-D-glucopyranosyljoxy $\}$-23-hydroxytirucall-7-ene-3,21-dione $\quad(=(3 \beta, 4 \beta, 13 \alpha, 14 \beta$, $17 \alpha, 20 \mathrm{~S}, 23 \mathrm{R}, 24 \mathrm{~S})$-24-(Acetyloxy)-29-f( $\mathrm{O}-\beta$-deoxy- $\alpha-\mathrm{L}-$ mannopyranosyl-( $1 \rightarrow 6$ )-O-[ $\beta$-d-glucopyranosyl$(1 \rightarrow 3)]-\beta$-D-glucopyranosylloxy]-21,25-epoxy-23-hydroxylanost-7-ene-3,21-dione; 4): White powder. $[\alpha]_{\mathrm{D}}^{22}=-58.9(c=0.15, \mathrm{MeOH})$. IR (KBr): 3430, 2936, 1748, 1706. ${ }^{1} \mathrm{H}-$ and ${ }^{13} \mathrm{C}$-NMR: Tables 1 and 2. FAB-MS (glycerol; neg.): 1013 ( $\left.100,[M-H]^{-}\right)$. HR-ESI-MS: $1013.4946\left(\mathrm{C}_{50} \mathrm{H}_{77} \mathrm{O}_{21}^{-}\right.$, calc. 1013.4957).
(3 $\beta, 20 \mathrm{~S}, 23 \mathrm{R}, 24 \mathrm{~S})$-24-(Acetyloxy)-21,25-epoxy-29-\{\{O- $\beta$-D-glucopyranosyl-( $1 \rightarrow 3$ )-O-[ $\alpha$-L-rhamno-pyranosyl-( $1 \rightarrow 6$ )]- $\beta$-D-glucopyranosyl\}oxy $\}$-3,23-dihydroxytirucall-7-en-21-one $\quad(=(3 \beta, 4 \beta, 13 \alpha, 14 \beta$, $17 \alpha, 20 \mathrm{~S}, 23 \mathrm{R}, 24 \mathrm{~S})$-24-(Acetyloxy)-29-\{\{O-6-deoxy- $\alpha-\mathrm{L}-$ mannopyranosyl-( $1 \rightarrow 6$ )-O-[ $\beta$-D-glucopyranosyl( $1 \rightarrow 3$ )]- $\beta$-D-glucopyranosylfoxy]-21,25-epoxy-3,23-dihydroxylanost-7-en-21-one; 5): White powder. $[\alpha]_{\mathrm{D}}^{22}=-37.8(c=0.15, \mathrm{MeOH})$. IR (KBr): 3424, 2933, 1749, 1638. ${ }^{1} \mathrm{H}-\mathrm{and}{ }^{13} \mathrm{C}-\mathrm{NMR}$ : Tables 1 and 2. FAB-MS (glycerol): $1015\left(100,[M-H]^{-}\right)$. HR-ESI-MS: $1015.5097\left(\mathrm{C}_{50} \mathrm{H}_{79} \mathrm{O}_{21}^{-}\right.$; calc. 1015.5113).

Acid Hydrolysis of 2-5. Compounds $\mathbf{2 - 5}(15 \mathrm{mg}$ each $)$ were refluxed with $10 \% \mathrm{HCl} / \mathrm{MeOH}(20 \mathrm{ml})$ at $80^{\circ}$ for 6 h . After cooling, the mixture was concentrated and the residue partitioned with $\mathrm{AcOEt} / \mathrm{H}_{2} \mathrm{O}$. The sugars in this mixture were identified as glucose and rhamnose by TLC comparison (MeCOEt/ ${ }^{i} \mathrm{PrOH} / \mathrm{Me}_{2} \mathrm{CO} / \mathrm{H}_{2} \mathrm{O} 20: 10: 7: 6$ ). Separation of the residue of the $\mathrm{H}_{2} \mathrm{O}$ layer was performed by prep. TLC ( $4 \times$ elution with $\mathrm{CHCl}_{3} / \mathrm{MeOH} / \mathrm{H}_{2} \mathrm{O} 70: 30: 1$ ): D-glucose ( $R_{\mathrm{f}} 0.50$ ) and L-rhamnose ( $R_{\mathrm{f}} 0.67$ ), with positive values of the specific rotation $\left([\alpha]_{\mathrm{D}}=19.2,21.5,24.6\right.$, and 24.0, and $[\alpha]_{\mathrm{D}}=26.5,25.0,21.5$, and 21.6 , resp.) The residue of the AcOEt layer was analyzed by HP-TLC (silica gel $G F_{254}$ plate, $\mathrm{CHCl}_{3} /$ $\mathrm{Me}_{2} \mathrm{CO} 3: 1$ and petroleum ether/ $\mathrm{CHCl}_{3} 3: 1$ ): Several decomposition products.

## REFERENCES

[1] H. Greger, T. Pacher, B. Brem, M. Bacher, O. Hofer, Phytochemistry 2001, 57, 57.
[2] Yunnan Institute of Botany, 'Flora of Yunnanica, Tomus 1', Science Press, Beijing, 1977, p. 214.
[3] S. H. Qi, D. G. Wu, L. Chen, Y. B. Ma, X. D. Luo, J. Asian Nat. Prod. Res. 2003, 5, 215; S. H. Qi, D. G. Wu, L. Chen, Y. B. Ma, X. D. Luo, Tetrahedron 2003, 59, 4193; S. H. Qi, D. G. Wu, L. Chen, Y. B. Ma, X. D. Luo, J. Agric. Food Chem. 2003, 51, 6949.
[4] W. R. Chan, D. R. Taylor, T. H. Yee, J. Chem. Soc., C 1970, 311.
[5] D. A. G. Cortez, P. C. Vieira, J. B. Fernandes, G. F. G. F. da Silva, A. G. Ferreira, Phytochemistry 1992, 31, 625.
[6] X. D. Luo, S. H. Wu, Y. B. Ma, D. G. Wu, Phytochemistry 2000, 54, 801.
[7] J. Eskander, C. Lavaud, S. M. Abdel-khalik, H. S. M. Soliman, I. I. Mahmoud, C. Long, J. Nat. Prod. 2005, 68, 832.
[8] H. Itokawa, E. Kishi, H. Morita, K. Takeya, Chem. Pharm. Bull. 1992, 40, 1053.
[9] X. H. Cai, X. D. Luo, J. Zhou, X. J. Hao, Org. Lett. 2005, 7, 2877.

