# Tetranortriterpenoids from Clausena excavata 

by Hong Ping He ${ }^{\mathrm{a}}$ ), Jian Xin Zhang ${ }^{\mathrm{b}}$ ), Yue Mao Shen $\left.{ }^{\mathrm{a}}\right)^{\mathrm{b}}$ ), Yi Neng He ${ }^{\mathrm{a}}$ ), Chang Xiang Chen ${ }^{\mathrm{a}}$ ), and Xiao Jiang Hao $\left.{ }^{* a}\right)^{b}$ )<br>${ }^{\text {a }}$ ) Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, P.R. China<br>${ }^{\text {b }}$ ) The Key Laboratory of Chemistry for Natural Products of Guizhou Province and the Chinese Academy of Sciences, Guiyang 550002, Guizhou, P.R. China


#### Abstract

Five new tetranortriterpenoids, $(11 \beta)$-21,23-dihydro-11,21-dihydroxy-23-oxoobacunone ( $=21,23$-dihydro-21-hydroxy-23-oxozapoterin; 2), ( $11 \beta$ )-21,23-dihydro-11,23-dihydroxy-21-oxoobacunone ( $=21,23$-dihydro-23-hydroxy-21-oxozapoterin; 3), ( $1 \alpha, 11 \beta$ )-1,2,21,23-tetrahydro-1,11,23-trihydroxy-21-oxoobacunone ( $=21,23$-di-hydro-23-hydroxy-21-oxoclausenarin; 4), ( $1 \alpha, 11 \beta$ )-23-ethoxy-1,2,21,23-tetrahydro-1,11-dihydroxy-21-oxoobacunone ( $=23$-ethoxy-21,23-dihydro-21-oxoclausenarin; 5); (11 $\beta$ )-1,2,21,23-tetrahydro-11,23-dihydroxy-21-oxoobacunoic acid; 6), were isolated from the aerial part of Clausena excavata Burm. F. (Rutaceae). All compounds possessed 3,4 -seco skeletons. Their structures were established by spectroscopic studies. Tetranortriterpenoids with a 4-hydroxybut-2-eno-4-lactone moiety are rarely found in the genus Clausena.


1. Introduction. - Clausena excavata Burm. F. (Rutaceae) is a bush growing in Xishuangbanna, Yunnan Province, P.R. China. Leaves and barks of this plant have been used in folk medicines for the treatment of dysentery, enteritis, and urethra infection [1]. Previous research revealed that this plant mainly contains alkaloids [2-4] and Oterpenoidal coumarins [5-10]. This paper describes the isolation and structure elucidation of the five new tetranortriterpenoids $2-6$ together with a known one, zapoterin $\left.^{1}\right)\left(=(11 \beta)\right.$-11-hydroxyobacunone $\left.\left.{ }^{2}\right) ; \mathbf{1}\right)$. Their structures were determined by spectroscopic analysis, especially 2D NMR experiments.

In general, an equilibrium between the $\alpha$ - and $\beta$-hydroxy isomers at $\mathrm{C}(21)$ or $\mathrm{C}(23)$ was suggested to be the cause of the abnormality observed in the ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}-\mathrm{NMR}$ spectra [11-15], especially in the ${ }^{13} \mathrm{C}$-NMR spectra of tetranortriterpenoids like $2-4$ and 6 having a 4 -hydroxybut-2-eno-4-lactone residue. Our experiments indicated that it was possible to obtain normal ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}-\mathrm{NMR}$ spectra for such tetranortriterpenoids by acetylation or methylation of the hydroxy group at the butenolactone residue such as in 2a or 2c, respectively (Fig.).
2. Results and Discussion. - The aerial parts of C. excavata were extracted with $90 \%$ EtOH . The EtOH extract was successively chromatographed over silica gel and Sephadex LH-20 to afford the six compounds 1-6.

[^0]


| 170 | 160 | 150 | 140 | 130 | 120 | 110 | 100 | 90 | 80 | ppm |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |





Compound 2, an amorphous powder, was determined to have the molecular formula $\mathrm{C}_{26} \mathrm{H}_{30} \mathrm{O}_{10}$ based on the high-resolution EI-MS peak at $\mathrm{m} / \mathrm{z} 502.1875$ ( $\mathrm{M}^{+}$, calc. 502.1839). The IR ( KBr ) data suggested the presence of carbonyl $\left(1750,1715 \mathrm{~cm}^{-1}\right)$ and OH ( $3487 \mathrm{~cm}^{-1}$ ) groups. The ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (Tables 1 and 2, resp.), ${ }^{1} \mathrm{H},{ }^{1} \mathrm{H}$ COSY, and ${ }^{1} \mathrm{H},{ }^{13} \mathrm{C}$-HMBC experiments, and the comparison with the NMR data of $\mathbf{1}$ [16][17] established 2 to be 21,23-dihydro-21-hydroxy-23-oxozapoterin ${ }^{1}$ ) $(=(11 \beta)$ -21,23-dihydro-11,21-dihydroxy-23-oxoobacunone ${ }^{2}$ )).

Table 1. ${ }^{1} H$-NMR Data for Compounds 2-6

|  | $\mathbf{2}^{\text {a }}{ }^{\text {c }}$ ) | $3^{\text {a }}{ }^{\text {c }}$ ) | $\left.4^{\text {b }}\right)^{\text {c }}$ ) | $5^{\text {b }}{ }^{\text {c }}$ ) | $\left.6^{\text {b }}\right)^{\text {c }}$ ) |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 1 or $2 \mathrm{H}-\mathrm{C}(1)$ | $\begin{aligned} & 6.79 \\ & (d, J=12.0) \end{aligned}$ | $6.61(d, J=12.0)$ | $5.52(d, J=7.1)$ | $\begin{aligned} & 5.49 \\ & (d, J=7.0,1 \mathrm{H}) \end{aligned}$ | $\begin{aligned} & 1.1-1.30 \\ & (m, 2 \mathrm{H}) \end{aligned}$ |
| 1 or $2 \mathrm{H}-\mathrm{C}(2)$ | $\begin{aligned} & 5.90 \\ & (d, J=12.0) \end{aligned}$ | $5.87(d, J=12.0)$ | $\begin{aligned} & 3.50 \\ & (d d, J=15.6,7.1) \\ & 3.78(d, J=15.6) \end{aligned}$ | $\begin{aligned} & 3.49 \\ & (d d, J=5.4,7.0) \\ & 3.74(d, J=15.4) \end{aligned}$ | $\begin{aligned} & 2.45-2.85 \\ & (m, 2 \mathrm{H}) \end{aligned}$ |
| $1 \mathrm{H}-\mathrm{C}(5)$ | $\begin{aligned} & 2.72 \\ & (d d, J=14.1,4.8) \end{aligned}$ | $\begin{aligned} & 2.71 \\ & (d d, J=14.1,4.8) \end{aligned}$ | $\begin{aligned} & 3.02 \\ & (d d, J=14.2,4.9) \end{aligned}$ | $\begin{aligned} & 3.02 \\ & (d d, J=14.1,4.9) \end{aligned}$ | $\begin{aligned} & 2.66 \\ & (d d, J=14.3,5.6) \end{aligned}$ |
| $2 \mathrm{H}-\mathrm{C}(6)$ | $\begin{aligned} & 2.29 \\ & (d d, J=14.1,4.8) \\ & 3.14(t, J=14.1) \end{aligned}$ | $\begin{aligned} & 2.29 \\ & (d d, J=14.1,4.8) \\ & 3.12(t, J=14.1) \end{aligned}$ | $\begin{aligned} & 2.78 \\ & (d d, J=14.2,4.9) \\ & 3.16(t, J=14.2) \end{aligned}$ | $\begin{aligned} & 2.74 \\ & (d d, J=14.1,4.9) \\ & 3.16(t, J=14.1) \end{aligned}$ | $\begin{aligned} & 2.45 \\ & d d, J=14.3,5.6) \\ & 2.77(d, J=14.3) \end{aligned}$ |
| $1 \mathrm{H}-\mathrm{C}(9)$ | 1.92 (s) | 1.91 (s) | 2.69 (s) | 2.67 ( $s$ ) | 2.59 (s) |
| $1 \mathrm{H}-\mathrm{C}(11)$ | 4.58 (br.s) | 4.49 (br.s) | 4.81 (br.s) | $4.79(d, J=6.2)$ | 4.33 (br.s) |
| $2 \mathrm{H}-\mathrm{C}(12)$ | 1.76 ( $d, J=14.9$ ) | $\begin{aligned} & 1.63 \\ & (d d, J=14.7,6.4) \end{aligned}$ | 2.02 (m) | 1.70-1.85 (m) | $\begin{aligned} & 2.50-2.85 \\ & (m, 2 \mathrm{H}) \end{aligned}$ |
|  | $\begin{aligned} & 2.05 \\ & (d d, J=14.9,4.2) \end{aligned}$ | $1.86(d, J=14.7)$ | 2.25 (m) | 2.50-2.65 (m) |  |
| $1 \mathrm{H}-\mathrm{C}(15)$ | 3.79 (s) | 3.78 (s) | 4.36 (s) | 4.39 (s) | 4.27 (s) |
| $1 \mathrm{H}-\mathrm{C}(17)$ | 5.22 (br.s) | 5.26 (br.s) | 5.95 (br.s) | 5.91 (s) | 5.97 (br.s) |
| $\mathrm{Me}(18)$ | 0.93 (s, 3 H ) | 0.98 (s, 3 H ) | 1.49 (s, 3 H) | 1.40 (s, 3 H$)$ | 1.45 (s, 3 H$)$ |
| $\mathrm{Me}(19)$ | 1.69 (s, 3 H) | 1.66 (s, 3 H ) | 1.96 (s, 3 H ) | 1.93 (s, 3 H$)$ | 1.95 ( $s, 3 \mathrm{H}$ ) |
| $1 \mathrm{H}-\mathrm{C}(21)$ | 6.04 (br.s) | - | - | - | - |
| $1 \mathrm{H}-\mathrm{C}(22)$ | 6.25 (br.s) | 7.53 (br.s) | 7.81 (br.s) | 7.65 (s) | 7.82 (br.s) |
| $1 \mathrm{H}-\mathrm{C}(23)$ | - | 6.22 (br.s) | 6.55 (br.s) | 6.05 (s) | 6.57 (br.s) |
| Me (28) | 1.33 (s, 3 H) | 1.33 (s, 3 H) | 1.33 (s, 3 H) | 131 (s, 3 H) | 1.42 (s, 3 H) |
| Me (29) | 1.48 (s, 3 H) | 1.44 (s, 3 H) | 1.58 (s, 3 H ) | 1.59 (s, 3 H) | 1.72 (s, 3 H ) |
| $\mathrm{Me}(30)$ | 1.47 (s, 3 H) | 1.43 (s, 3 H ) | 1.88 (s, 3 H ) | 1.84 (s, 3 H ) | 1.85 (s, 3 H) |
| Others | - | - | - | $\begin{aligned} & 1.09 \\ & (t, J=7.0,3 \mathrm{H}) \\ & 3.62(q, J=7.0) \\ & 3.74(q, J=7.0) \end{aligned}$ | - |

${ }^{\text {a }}$ ) In $\left(\mathrm{D}_{6}\right)$ DMSO. ${ }^{\mathrm{b}}$ ) In $\mathrm{C}_{5} \mathrm{D}_{5} \mathrm{~N}$. ${ }^{\text {c }}$ ) Coupling constants in Hz .

The ${ }^{1} \mathrm{H}$-NMR spectra of $\mathbf{2}$ revealed the presence of 5 Me groups at $\delta 0.93,1.33,1.47,1.48$, and $1.69(5 s$, each 3 H ). In the ${ }^{13} \mathrm{C}$-NMR (Table 2) of 226 C -signals appeared: $5 \mathrm{Me}, 2 \mathrm{CH}_{2}, 9 \mathrm{CH}$, and 10 C . The ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}$-NMR, ${ }^{1} \mathrm{H},{ }^{1} \mathrm{H}$ COSY, and HMBC experiments showed that it was a derivative of zapoterin $\left.{ }^{1}\right)(\mathbf{1})$, whose ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}-\mathrm{NMR}$ data were consistent with those in [16] [17]. The differences between $\mathbf{2}$ and $\mathbf{1}$ arose from the furan ring. The structure of the furan ring in $\mathbf{2}$ was assigned by analyzing the ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}$-NMR data with the aid of HMQC and HMBC experiments. In the ${ }^{1} \mathrm{H}$-NMR spectrum, a pair of protons at $\delta 6.79$ and $5.90(d, J=12.0 \mathrm{~Hz}$, 1 H each) was attributed to a cis-disubstituted double bond of an $\alpha, \beta$-unsaturated lactone at $\mathrm{C}(1)$ and $\mathrm{C}(2)$, respectively. The carbonyl group at $\delta(\mathrm{C}) 207.6$ was attributed to $\mathrm{C}(7)=\mathrm{O}$ by means of ${ }^{1} \mathrm{H},{ }^{13} \mathrm{C}$ long-range

|  | $\mathbf{1}^{\text {a }}$ ) | $2^{\text {a }}$ ) | 2a ${ }^{\text {a }}$ ) | 2b ${ }^{\text {a }}$ ) | 2c ${ }^{\text {a }}$ ) | $3^{\text {a }}$ ) | 4 ${ }^{\text {b }}$ ) | $5^{\text {b }}$ ) | $6^{\text {b }}$ ) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathrm{CH}(1)$ or $\mathrm{CH}_{2}(1)$ | 157.0 | 156.2 | 155.3 | 155.2 | 161.7 | 156.3 | 72.8 | 72.7 | 36.4 |
| $\mathrm{CH}(2)$ or $\mathrm{CH}_{2}(2)$ | 120.8 | 120.6 | 121.2 | 121.3 | 118.7 | 120.5 | 35.8 | 35.7 | 42.4 |
| C(3) | 166.9 | 166.3 | 165.5 | 165.8 | 166.3 | 166.5 | 170.2 | 169.6 | 174.2 |
| C(4) | 84.1 | 83.9 | 83.9 | 83.9 | 71.9 | 83.8 | 85.0 | 84.6 | 73.9 |
| CH(5) | 55.5 | 55.2 | 54.5 | 54.8 | 57.8 | 55.1 | 51.8 | 52.4 | 55.9 |
| $\mathrm{CH}_{2}(6)$ | $39.9{ }^{\text {c }}$ ) | 39.4 | 39.0 | 39.2 | 37.2 | 39.5 | 39.7 | 39.5 | 39.7 |
| C (7) | 208.1 | 207.6 | 206.5 | 206.6 | 209.5 | 207.8 | 208.3 | 207.8 | 210.4 |
| C(8) | $51.2{ }^{\text {c }}$ ) | 51.1 | 51.3 | 51.0 | 50.8 | 50.9 | 52.6 | 51.5 | 52.2 |
| CH(9) | 49.7 | 49.2 | 47.5 | 47.7 | 47.3 | 49.3 | 47.2 | 48.8 | 47.0 |
| C(10) | $43.6{ }^{\text {c }}$ ) | 43.5 | 43.0 | 43.0 | 45.0 | 43.6 | 45.7 | 47.0 | 48.0 |
| CH(11) | 65.7 | 65.2 | 68.4 | 68.0 | 64.8 | 65.1 | 65.3 | 65.1 | 67.0 |
| $\mathrm{CH}_{2}(12)$ | $43.2{ }^{\text {c }}$ ) | 42.2 | 36.4 | 38.5 | 41.9 | 41.4 | 43.4 | 43.5 | 43.5 |
| C(13) | 36.0 | 36.1 | 36.0 | 36.1 | 35.9 | 36.3 | 37.6 | 37.4 | 37.1 |
| C(14) | 64.7 | 64.4 | 64.1 | 64.2 | 64.3 | 64.3 | 65.4 | 65.2 | 63.7 |
| CH(15) | 53.2 | 52.5 | 52.3 | 52.4 | 52.3 | 53.0 | 54.5 | 54.4 | 54.0 |
| C(16) | 167.7 | 166.5 | 166.4 | 166.4 | 166.3 | 166.6 | 167.8 | 167.8 | 167.7 |
| CH(17) | 78.0 | $78.4{ }^{\text {d }}$ ) | 78.3 | $78.2{ }^{\text {d }}$ ) | 78.3 | $75.3{ }^{\text {d }}$ ) | $76.6{ }^{\text {d }}$ ) | 76.5 | $76.8{ }^{\text {d }}$ ) |
| Me(18) | 20.0 | 19.6 | 20.0 | 19.4 | 19.5 | 19.2 | 20.1 | 20.0 | 20.1 |
| Me(19) | 18.2 | 18.1 | 17.7 | 17.5 | 16.5 | 18.0 | 17.8 | 16.6 | 18.5 |
| C(20) | 120.3 | $163.9{ }^{\text {d }}$ ) | 160.8 | $163.8{ }^{\text {d }}$ ) | 166.1 | $131.8{ }^{\text {d }}$ ) | $133.1{ }^{\text {d }}$ ) | 133.8 | $133.3{ }^{\text {d }}$ ) |
| $\mathrm{C}(21)$ or $\mathrm{CH}(21)$ | 141.7 | $98.3{ }^{\text {d }}$ ) | 92.7 | $98.0{ }^{\text {d }}$ ) | 102.9 | $170.1^{\text {d }}$ ) | $169.9{ }^{\text {d }}$ ) | 169.6 | $170.8^{\text {d }}$ ) |
| $\mathrm{CH}(22)$ | 110.4 | $122.0{ }^{\text {d }}$ ) | 122.9 | $122.2{ }^{\text {d }}$ ) | 122.8 | $153.5{ }^{\text {d }}$ ) | $153.8{ }^{\text {d }}$ ) | 151.0 | $153.9{ }^{\text {d }}$ ) |
| $\mathrm{C}(23)$ or $\mathrm{CH}(23)$ | 143.6 | $169.0{ }^{\text {d }}$ ) | 168.2 | $168.9{ }^{\text {d }}$ ) | 168.5 | $98.2{ }^{\text {d }}$ ) | $99.0{ }^{\text {d }}$ ) | 102.3 | $99.6{ }^{\text {d }}$ ) |
| $\mathrm{Me}(28)$ | 31.8 | 31.5 | 31.6 | 31.5 | 30.5 | 31.5 | 33.7 | 31.3 | 33.4 |
| Me (29) | 26.5 | 26.2 | 26.3 | 26.3 | 29.6 | 26.1 | 23.2 | 23.0 | 29.4 |
| Me (30) | 19.4 | 18.9 | 18.5 | 18.5 | 18.5 | 18.9 | 20.7 | 19.9 | 19.6 |
| Others | - | - | 169.7 (s) | 169.6 (s) | 51.1 (q) | - | - | 66.1 (t) | - |
|  |  |  | 168.5 (s) | 21.1 (q) |  |  |  | 13.7 (q) |  |
|  |  |  | 21.4 (q) |  |  |  |  |  |  |
|  |  |  | 21.1 (q) |  |  |  |  |  |  |

${ }^{\text {a }}$ ) In $\left(\mathrm{D}_{6}\right)$ DMSO. ${ }^{\text {b }}$ ) In $\mathrm{C}_{5} \mathrm{D}_{5} \mathrm{~N}$. ${ }^{\text {c }}$ ) Revised assignments are based on the HMQC, HMBC, and ${ }^{1} \mathrm{H},{ }^{1} \mathrm{H}$ COSY experiments. ${ }^{\mathrm{d}}$ ) Weak and broad signals.
correlations between $\delta(\mathrm{H}) 1.14(\mathrm{Me}(30))$ and $\delta(\mathrm{C}) 49.2(\mathrm{C}(9))$, $51.1(\mathrm{C}(10)), 64.4(\mathrm{C}(14))$, and 207.6. In the ${ }^{1} \mathrm{H},{ }^{1} \mathrm{H}$ COSY plot, $\delta 4.58(d, J=4.2 \mathrm{~Hz})$ showed linear coupling with $\delta 2.07(d d, J=12.0,4.2 \mathrm{~Hz}, \mathrm{H}-\mathrm{C}(12))$, establishing that $\delta(\mathrm{C}) 65.2(\mathrm{~d})$ was arising from $\mathrm{C}(11)$. In the ${ }^{13} \mathrm{C}$-NMR spectrum, $\delta(\mathrm{C}) 64.4(s)$ and $52.5(d)$ were attributed to an epoxy ring between $\mathrm{C}(14)$ and $\mathrm{C}(15)$, which was supported by the ${ }^{1} \mathrm{H},{ }^{13} \mathrm{C}$ long-range correlations between $\delta(\mathrm{H}) 3.79$ and $\delta(\mathrm{C}) 51.1(\mathrm{C}(8)), 64.4(\mathrm{C}(14))$, and 166.5 . Thus, the ${ }^{13} \mathrm{C}$ - and ${ }^{1} \mathrm{H}$-chemical shifts of the rings A, B, C, and D of compounds $\mathbf{2}$ and $\mathbf{1}$ correspond to each other, so that the structures of these rings are identical, including their relative configurations. As for ring E of $\mathbf{2}$, the ${ }^{13} \mathrm{C}$-NMR signals at $\delta(\mathrm{C}) 98.3$ $(d), 122.0(d), 163.9(s)$, and $169.0(s)$ revealed the presence of a 4-hydroxybut-2-eno-4-lactone function. The signals at $\delta(\mathrm{C}) 122.0(d)$, and $169.0(s)$ were assigned to $\mathrm{C}(22)$ and $\mathrm{C}(23)$, resp. In the HMBC, the long-range correlations between $\delta(\mathrm{H}) 5.22(\mathrm{H}-\mathrm{C}(17))$ and $\delta(\mathrm{C}) 163.9(s)$ suggested that the olefinic C -atom at $\delta(\mathrm{C}) 163.9$ $(s)$ was $\mathrm{C}(20)$, which was in agreement with the conjugative effect of the $\mathrm{C}(23)$ carboxylate moiety. Then, the hemiacetal C -atom at $\delta(\mathrm{C}) 98.3(d)$ was $\mathrm{C}(21)$. The signals of $\mathrm{C}(17), \mathrm{C}(20), \mathrm{C}(21), \mathrm{C}(22)$, and $\mathrm{C}(23)$ appeared broad in the ${ }^{13} \mathrm{C}$-NMR spectra (see Fig.)), due to the equilibrium between the $\alpha$ - and $\beta$-hydroxy isomers at $\mathrm{C}(21)$. Other limonoids with a 4-hydroxybut-2-eno-4-lactone function and an OH group either at $\mathrm{C}(21)$ or at $\mathrm{C}(23)$ also were mixtures of their $\alpha$ - and $\beta$-hydroxy isomers [11-15]. Another noteworthy phenomenon was that the signal of $\mathrm{C}(4)$ at $\delta(\mathrm{C}) 83.9(s)$ was strong, which was not indicated in previous reports.

Compounds 2a and 2b were the di- and monoacetates of 2, and 2c was the methylester of (11 $\beta$ )-21,23-dihydro-11-hydroxy-21-methoxy-23-oxoobacunonic acid ${ }^{3}$ ). The signals of $C(17), C(20), C(21), C(22)$, and $C(23)$ were broad in $\mathbf{2}$ and $\mathbf{2 b}$, but turned out normal in 2a and 2c (Fig.).

Compound 3 was obtained as an amorphous powder. Its IR spectrum showed absorptions of $\mathrm{OH}\left(3498 \mathrm{~cm}^{-1}\right)$. The ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}$-NMR (Tables 1 and 2, resp.) and HR-FAB-MS data (negative mode) were consistent with the molecular formula $\mathrm{C}_{26} \mathrm{H}_{30} \mathrm{O}_{10}$. The ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}-\mathrm{NMR}, \mathrm{HMQC}, \mathrm{HMBC}$, and ${ }^{1} \mathrm{H},{ }^{1} \mathrm{H}$-COSY experiments and the comparison with the NMR data of $\mathbf{2}$ established $\mathbf{3}$ to be 21,23-dihydro-23-hydroxy-21oxozapoterin $\left.^{1}\right)\left(=(11 \beta)\right.$-21,23-dihydro-11,23-dihydroxy-21-oxoobacunone $\left.\left.{ }^{2}\right)\right)$.

The ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}$-NMR spectra of $\mathbf{3}$ revealed the presence of 5 Me at $\delta(\mathrm{H}) 0.98,1.33,1.43,1.44$, and $1.66(5 s$, each 3 H ); cis-positioned olefinic protons at $\delta(\mathrm{H}) 6.81$ and $5.87(d, J=12.0 \mathrm{~Hz}, 1 \mathrm{H}$ each $), 4 \mathrm{CH}-\mathrm{O}$ at $\delta(\mathrm{H})$ 4.49 (br. $s, \mathrm{H}-\mathrm{C}(11)), 3.78(s, \mathrm{H}-\mathrm{C}(15)), 5.23$ (br. $s, \mathrm{H}-\mathrm{C}(17)$ ), 6.22 (br. $s, \mathrm{H}-\mathrm{C}(23)$ ); $\delta(\mathrm{C}) 65.1$ ( $\mathrm{C}(11)$ ), 53.0 (C(15)), $75.3(\mathrm{C}(17)), 98.2(\mathrm{C}(13))$. The ${ }^{13} \mathrm{C}$-NMR spectra showed 26 C -signals: $5 \mathrm{Me}, 2 \mathrm{CH}_{2}, 9 \mathrm{CH}$, and 10 C . The ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}$-NMR data were similar to those of $\mathbf{2}$, except for the signals of the furan ring, suggesting that $\mathbf{3}$ and 2 have the same rings A-D. This was supported by the HMBC and ${ }^{1} \mathrm{H},{ }^{1} \mathrm{H}$-COSY experiments of $\mathbf{3}$. The ${ }^{13} \mathrm{C}$-NMR signals at $\delta(\mathrm{C}) 98.2(\mathrm{~d}), 131.8(\mathrm{~s}), 153.5(\mathrm{~d})$, and $170.1(s)$ showed the presence of a 4-hydroxybut-2-eno-4-lactone function. The signal at $\delta(\mathrm{C}) 170.1(s)$ was assigned to the $\alpha, \beta$-unsaturated $\gamma$-lactone carbonyl at $\mathrm{C}(21)$, and the hemiacetal C -atom at $\delta(\mathrm{C}) 98.2(d)$ was attributed to $\mathrm{C}(23)$. The remaining two C -signals at $\delta(\mathrm{C})$ $131.8(s)$ and $153.5(d)$ were ascribed to the olefinic C-atoms $\mathrm{C}(20)$ and $\mathrm{C}(22)$, resp., which was consistent with the $\alpha, \beta$-conjugative effect of the $\mathrm{C}(21)$ carboxylate moiety. In compound $\mathbf{3}$, the signal of $\mathrm{C}(11)$ at $\delta(\mathrm{C}) 75.3$ was broad, and the signals of $\mathrm{C}(20), \mathrm{C}(21), \mathrm{C}(22)$, and $\mathrm{C}(23)$ were split.

Compound 4 was obtained as a powder. Its IR spectrum showed absorptions of OH ( $3498 \mathrm{~cm}^{-1}$ ), and its ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (Tables 1 and 2, resp.) and HR-FAB-MS (negative mode) were consistent with the molecular formula $\mathrm{C}_{26} \mathrm{H}_{32} \mathrm{O}_{11}$. The ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}$-NMR, HMQC, HMBC, and ${ }^{1} \mathrm{H},{ }^{1} \mathrm{H}$-COSY experiments and the comparison with those of $\mathbf{3}$ and clausenarin [16] established 4 to be 21,23-dihydro-23-hydroxy-21oxoclausenarin $\left.^{4}\right) \quad(=(1 \alpha, 11 \beta)-1,2,21,23$-tetrahydro-1,11,23-trihydroxy-21-oxoobacunone $\left.{ }^{2}\right)$ ).

The ${ }^{1} \mathrm{H}$-NMR of $\mathbf{4}$ revealed the presence of 5 Me at $\delta(\mathrm{H}) 1.33,1.49,1.58,1.88$, and 1.96 ( $5 s$, each 3 H ). The ${ }^{13} \mathrm{C}$-NMR showed 26 C -signals: $5 \mathrm{Me}, 3 \mathrm{CH}_{2}, 8 \mathrm{CH}$ (including $5 \mathrm{CH}-\mathrm{O}$ at $\delta 54.5(\mathrm{C}(15)), 65.3(\mathrm{C}(11))$ ) 72.8 $(\mathrm{C}(17))$, $76.6(\mathrm{C}(1))$, and $99.0(\mathrm{C}(23)))$, and 10 C . Compound 4 has the same $\mathrm{A}-\mathrm{D}$ ring structure as clausenarin [16] according to its ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}$-NMR. The ${ }^{13} \mathrm{C}$-NMR signals at $\delta(\mathrm{C}) 99.0(d), 131.1(s), 153.8(d)$, and $169.9(s)$ showed the presence of the same 4-hydroxybut-2-eno-4-lactone function as in 3 .

The HR-EI-MS of $\mathbf{5}$ gave its molecular formula $\mathrm{C}_{28} \mathrm{H}_{36} \mathrm{O}_{11}$. Based on the similarities of the NMR spectra of $\mathbf{5}$ and $\mathbf{4}$ (Tables 1 and 2), the structure of $\mathbf{5}$ was determined to be 23-ethoxy-21,23-dihydro-21-oxoclausenarin $\left.{ }^{4}\right)(=1 \alpha, 11 \beta)$-23-ethoxy-1,2,21,23-tetrahy-dro-1,11-dihydroxy-21-oxoobacunone ${ }^{2}$ ).

The ${ }^{1} \mathrm{H}$-NMR data of $\mathbf{5}$ revealed the presence of 6 Me at $\delta(\mathrm{H}) 1.09(t), 1.31(s), 1.40(s), 1.59(s), 1.84(s)$, and $1.93(s)$. The ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}$-NMR spectra of $\mathbf{5}$ showed similarities with those of $\mathbf{4}$, except for the presence of

[^1]an EtO group $(\delta(\mathrm{H}) 3.74(m, 1 \mathrm{H}), 3.62(q, J=7.0 \mathrm{~Hz}, 1 \mathrm{H}), 1.09(t, J=7.0 \mathrm{~Hz}, 3 \mathrm{H}) ; \delta(\mathrm{C}) 66.1(t), 13.7(q))$. The chemical shift of $\mathrm{C}(23)$ of $\mathbf{5}$ was shifted downfield to $\delta(\mathrm{C}) 102.3(d)$ compared with that of $\mathbf{4}$, suggesting that the EtO group was linked at $\mathrm{C}(23)$. This was also confirmed by the HMBC experiment.

The HR-FAB-MS (negative mode) of 6 showed its molecular formula to be $\mathrm{C}_{26} \mathrm{H}_{34} \mathrm{O}_{11}$. The ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}$-NMR spectra (Tables 1 and 2, resp.) and their comparison with the data of $\mathbf{3}$ revealed the structure of $\mathbf{6}$ to be ( $11 \beta$ )-1,2,21,23-tetrahydro-11,23-dihydroxy-21-oxoobacunoic acid ${ }^{3}$ ).

The ${ }^{13} \mathrm{C}$-NMR of $\mathbf{6}$ revealed the presence of 26 C -atoms: $5 \mathrm{Me}, 4 \mathrm{CH}_{2}, 7 \mathrm{CH}$, and 10 C (including $2 \mathrm{C}-\mathrm{O}$ at $\delta 63.7$, and 73.9). On the basis of the similarities between the ${ }^{13} \mathrm{C}$-NMR spectra of $\mathbf{6}$ and $\mathbf{3}$, it was concluded that rings B-E of $\mathbf{6}$ were the same as those of $\mathbf{3}$. The difference between $\mathbf{6}$ and $\mathbf{3}$ was in ring A. The signals of the cisdisubstitued double bond were absent in $\mathbf{6}$ and replaced by two $\mathrm{CH}_{2}$ at $\delta(\mathrm{C}) 36.4$ and 42.4 , and the resonances of $\mathrm{C}(4)$ and $\mathrm{C}(3)$ were shifted upfield to 73.9 and 174.2 ppm , resp., consistent with a $\mathrm{C}(1) / \mathrm{C}(2)$ saturated acid.

## Experimental Part

General. UV Spectra: $U V$-210A spectrophotometer; $\lambda_{\text {max }}$ in nm. IR Spectra: KBr pellets; Perkin-Elmer 577 spectrophotometer; in $\mathrm{cm}^{-1}$. NMR Spectra: 1D, Bruker AM-400 spectrometer; 2D, Bruker DRX-500 spectrometer; $\delta$ in ppm rel. to $\mathrm{SiMe}_{4}(=0 \mathrm{ppm})$, $J$ in Hz. MS: VG Autospec- 3000 spectrometer.

Plant Material. The aerial parts of Clausena excavata Burm. F. were collected in Xishuangbanna, Yunnan, China. A voucher specimen of this plant was deposited in the Kunming Institute of Botany, Kunming, China.

Extraction and Isolation. The powdered aerial part of C. excavata ( 6.0 kg ) was extracted three times with $90 \% \mathrm{EtOH}(12 \mathrm{l})$ under reflux for 8 h each time. The extract ( 620 g ) was chromatographed (silica gel, $\mathrm{CHCl}_{3}$, $\mathrm{CHCl}_{3} / \mathrm{AcOEt}$, AcOEt , and MeOH , successively). The $\mathrm{CHCl}_{3} / \mathrm{AcOEt}$ eluate $(60 \mathrm{~g})$ was further subjected to column chromatography (silica gel, gradient of petroleum ether/AcOEt $7: 3,6: 4,1: 1,4: 6$ and $3: 7$ ). The last two fractions (petroleum ether/AcOEt $4: 6$ and $3: 7$ ) were combined and chromatographed over silica gel and Sephadex LH-20 to give $\mathbf{1}(2.618 \mathrm{~g}), \mathbf{2}(189 \mathrm{mg}), \mathbf{3}(12 \mathrm{mg}), \mathbf{4}(15 \mathrm{mg}), 5(9 \mathrm{mg})$, and $\mathbf{6}(10 \mathrm{mg})$.
(11 $\beta$ )-21,23-Dihydro-11,21-dihydroxy-23-oxoobacunone ${ }^{2}$ ) (2). Amorphous powder. $[\alpha]_{\mathrm{D}}^{24}=-32.4$ ( $c=$ $0.55, \mathrm{MeOH})$. UV: 210 . IR: 3487, 2992, 2947, 1750, 1713, 1461, 1433, 1399, 1378, 1275, 1230, 1138, 1119, 1049, 978, 905, $843 .{ }^{1} \mathrm{H}-\mathrm{NMR}$. Table 1. ${ }^{13} \mathrm{C}$-NMR: Table 2. EI-MS: 502 (2), 484 (3), 458 (6), 440 (11), 425 (8), 387 (10), 251 (15), 136 (74), 91 (73), 55 (100). HR-EI-MS: $502.1876\left(\mathrm{C}_{26} \mathrm{H}_{30} \mathrm{O}_{10}^{+}\right.$; calc. 502.1839).

Compounds 2a and 2b. Obacunone $2(60 \mathrm{mg})$, pyridine ( 4 ml ), and $\mathrm{Ac}_{2} \mathrm{O}(2 \mathrm{ml})$ were stirred for 48 h . Usual workup and chromatography (silica gel) gave $\mathbf{2 a}(30 \mathrm{mg})$ and $\mathbf{2 b}(24 \mathrm{mg})$.
(11 $\beta$ )-11,21-Diacetoxy-21,23-dihydro-23-oxoobacunone ${ }^{2}$ ) (2a): ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\left(\mathrm{D}_{6}\right) \mathrm{DMSO}, 400 \mathrm{MHz}\right): 1.03$, $1.33,1.42,1.43,1.50,2.07,2.09$ ( $7 \mathrm{~s}, 7 \mathrm{Me}$ ); $3.93(s, \mathrm{CH}(15)) ; 5.58$ ( $d, J=6.7, \mathrm{CH}(11)$ ); 5.28 (br. $s, \mathrm{CH}(17)$ ); 5.93 $(d, J=12.0, \mathrm{CH}(2)) ; 6.51(s, \mathrm{CH}(22)) ; 6.83(d, J=12.0, \mathrm{CH}(1)) ; 6.89(s, \mathrm{CH}(21)) .{ }^{13} \mathrm{C}-\mathrm{NMR}:$ Table 2. EI-MS: 586 (1), 485 (5), 468 (5), 426 (9), 136 (100), 108 (56).
(11 $\beta$ )-11-Acetoxy-21,23-dihydro-21-hydroxy-23-oxoobacunone $\left.{ }^{2}\right)(\mathbf{2 b}):{ }^{1} \mathrm{H}-\mathrm{NMR}\left(\left(\mathrm{D}_{6}\right) \mathrm{DMSO}, 400 \mathrm{MHz}\right)$ : $0.98,1.33,1.41,1.42,1.46,2.07(6 s, 6 \mathrm{Me}) ; 3.89(s, \mathrm{CH}(15)) ; 5.67$ (br. $s, \mathrm{CH}(11)) ; 5.15$ (br. $s, \mathrm{CH}(17)) ; 5.93(d, J=$ $12.0, \mathrm{CH}(2)) ; 6.01$ (br. $s, \mathrm{CH}(21)$ ); 6.25 (br. $s, \mathrm{CH}(22)$ ); $6.83(d, J=12.0, \mathrm{CH}(1)) .{ }^{13} \mathrm{C}-\mathrm{NMR}:$ Table 2. EI-MS: 544 (3), 486 (5), 469 (4), 443 (17), 425 (11), 136 (100), 108 (57).

Methyl (11 $\beta$ )-21,23-Dihydro-11-hydroxy-21-methoxy-23-oxoobacunonate ${ }^{3}$ ) (2c). Obacunone $2(50 \mathrm{mg})$ and $3 \%$ aq. $\mathrm{H}_{2} \mathrm{SO}_{4}$ soln. ( 10 ml ) were refluxed for 3 h . Usual workup and chromatography (silica gel) afforded $\mathbf{2 c}$ $(28 \mathrm{mg}) .{ }^{1} \mathrm{H}-\mathrm{NMR}\left(\left(\mathrm{D}_{6}\right) \mathrm{DMSO}, 400 \mathrm{MHz}\right): 0.95,1.08,1.09,1.41,1.65(5 \mathrm{~s}, 5 \mathrm{Me}) ; 3.50(s, 1 \mathrm{MeO}) ; 3.67$ $(s, 1 \mathrm{MeO}) ; 4.97(s, \mathrm{CH}(17)) ; 5.13$ (br. $s, \mathrm{CH}(21)) ; 5.90(d, J=16.0, \mathrm{CH}(2)) ; 6.00(s, \mathrm{CH}(22)) ; 6.95(d, J=16.0$, CH(1)). EI-MS: 548 (3), 530 (4), 446 (43), 431 (11), 107 (52), 59 (100).
(11 $\beta$ )-21,23-Dihydro-11,23-dihydroxy-21-oxoobacunone $\left.{ }^{2}\right)(3)$. Amorphous powder. $[\alpha]_{\mathrm{D}}^{25}=-5.0(c=0.45$, MeOH). UV: 214. IR: 3498, 2999, 2960, 1767, 1728, 1692, 1434, 1399, 1382, 1284, 1251, 1117, 1075, 1021, 986, 934, 682. ${ }^{1} \mathrm{H}$-NMR: Table $1 .{ }^{13} \mathrm{C}$-NMR: Table 2. EI-MS: 502 (1), 484 (2), 136 (86), 55 (100). FAB-MS (neg.): 501. HR-FAB-MS (neg.): $501.1708\left(\mathrm{C}_{26} \mathrm{H}_{29} \mathrm{O}_{10}^{+}\right.$; calc. 501.1761).
(1 $\alpha, 11 \beta)-1,2,21,23-T e t r a h y d r o-1,11,23$-trihydroxy-21-oxoobacunone ${ }^{2}$ ) (4). Amorphous powder. $[\alpha]_{\mathrm{D}}^{24}=$ $-49.6(c=0.63, \mathrm{MeOH}) . \mathrm{UV}: 203.5 . \operatorname{IR}: 3472,2992,2947,1749,1715,1630,1431,1399,1378,1279,1231$,

1118, 1028, 979, 937. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ : Table 1. ${ }^{13} \mathrm{C}-\mathrm{NMR}$ : Table 2. EI-MS: $520(1), 502\left(2,\left[M-\mathrm{H}_{2} \mathrm{O}\right]^{+}\right), 440(21), 422$ (49), $407(42), 165(59), 91(100)$. HR-EI-MS: $520.1940\left(\mathrm{C}_{26} \mathrm{H}_{32} \mathrm{O}_{11}^{+}\right.$; calc. 520.1945).
(1 $\alpha, 11 \beta$ )-23-Ethoxy-1,2,21,23-tetrahydro-1,11-dihydroxy-21-oxoobacunone ${ }^{2}$ ) (5). Amorphous powder. $[\alpha]_{\mathrm{D}}^{25}=-21.9(c=0.40, \mathrm{MeOH}) . \mathrm{UV}: 205 . \mathrm{IR}: 3487,2985,2944,1750,1717,1461,1432,1377,1277,1230,1119$, 1029, 979, 935. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ : Table 1. ${ }^{13} \mathrm{C}-\mathrm{NMR}$ : Table 2. EI-MS: $548(2), 530\left(10,\left[M-\mathrm{H}_{2} \mathrm{O}\right]^{+}\right), 472(20), 433(19)$, 415 (11), 136 (100). HR-EI-MS: $548.2263\left(\mathrm{C}_{28} \mathrm{H}_{36} \mathrm{O}_{11}^{+}\right.$; calc. 548.2258).
(11 $\beta$ )-1,2,21,23-Tetrahydro-11,23-dihydroxy-21-oxoobacunoic Acid ${ }^{3}$ ) (6). Amorphous powder. $[\alpha]_{\mathrm{D}}^{25}=$ $-53.8(c=0.40, \mathrm{MeOH})$. UV: 204.5. IR: 3471, 2978, 2940, 1745, 1731, 1380, 1270, 1207, 1140, 1024, $939,689$. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ : Table 1. ${ }^{13} \mathrm{C}-\mathrm{NMR}$ : Table 2. FAB-MS (neg.): 521. HR-FAB-MS (neg.): $521.1995\left(\mathrm{C}_{26} \mathrm{H}_{33} \mathrm{O}_{11}^{+}\right.$; calc. 521.2013).

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[^0]:    $\left.{ }^{1}\right) \quad$ Zapoterin $=(1 S, 3 \mathrm{a} S, 4 \mathrm{a} R, 4 \mathrm{~b} R, 6 \mathrm{a} R, 11 \mathrm{a} R, 11 \mathrm{~b} R, 12 S, 13 \mathrm{a} S)$-1-(furan-3-yl)-1,6a,7,11a,11b,12,13,13a-octahydro-12-hydroxy-4b,7,7,11a,13a-pentamethyloxirano[4,4a]-2-benzopyrano[6,5-g][2]benzoxepin3,5,9(2a $H, 4 \mathrm{~b} H, 6 H)$-trione.
    $\left.{ }^{2}\right)$ Obacunone $=(1 S, 3 \mathrm{a} S, 4 \mathrm{a} R, 4 \mathrm{~b} R, 6 \mathrm{a} R, 11 \mathrm{a} R, 11 \mathrm{~b} R, 13 \mathrm{a} S)$-1-(furan-3-yl)-1,6a,7,11a,11b,12,13,13a-octahydro-4b,7,7,11a,13a-pentamethyloxireno[4,4a]-2-benzopyrano[6,5-g][2]benzoxepin-3,5,9(3a $H, 4 \mathrm{~b} H, 6 H)$-trione.

[^1]:    $\left.{ }^{3}\right)$ Obacunonic acid $=(3 S, 3 \mathrm{a} S, 5 \mathrm{a} R, 6 R, 7 R, 9 \mathrm{a} R, 9 \mathrm{~b} R, 10 \mathrm{a} S)$-3-[3-(furan-3-yl)dodecahydro-7-(1-hydroxy-1-methyl-ethyl)-3a,6,9a-trimethyl-1,9-dioxonaphth[2,1-c]oxireno[ $d$ ]pyran-6-yl]prop-2-enoic acid.
    $\left.{ }^{4}\right) \quad$ Clausenarin $=(1 S, 3 \mathrm{a} S, 4 \mathrm{a} R, 4 \mathrm{~b} R, 6 \mathrm{a} R, 11 S, 1 \mathrm{a} R, 11 \mathrm{~b} R, 12 S, 13 \mathrm{a} S)$-1-(furan-3-yl)decahydro-11,12-dihydroxy-4b,7,7,11a,13a-pentamethyloxireno[4,4a]-2-benzopyrano[6,5-g][2]benzoxepin-3,5,9(3a $H, 4 \mathrm{~b} H, 6 H)$-trione.

