Chinese Chemical Letters Vol. 11, No. 1, pp. 57–60, 2000

Two New C_{29} Sterols from *Clerodendrum colebrookianum*

Hui YANG[1,2], Shuang Xi MEI[1], Bei JIANG[1], Zhong Wen LIN[1], Han Dong SUN[1]*

[1]Laboratory of Phytochemistry, Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming 650204
[2]Chemistry Department of the College of Life Science and Chemistry, Yunnan University, Kunming 650091

Abstract: From the aerial parts of *Clerodendrum colebrookianum* Walp., two new C_{29} sterols, colebrin A and colebrin B, were isolated, along with a known compound, clerosterol . The structures of the new compounds were elucidated on the basis of spectral evidence.

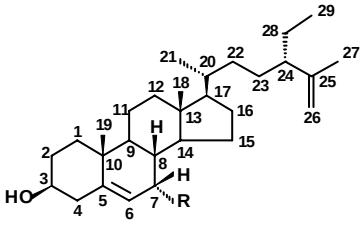
Keywords: *Clerodendrum colebrookianum*, Verbenaceae, C_{29} sterol, colebrin A, colebrin B.

Clerodendrum. colebrookianum Walp. is a traditional Chinese medicine, it possesses the functions of "expelling toxin by cooling, cooling blood to induce diuresis and purging heat"[1], and has been used as a remedy for hypertension in India[2]. The chemical investigation of this plant has been reported previously[2-8]. In continuation of our studies on the constituents of the plant, we examined the steroid constituents of the title species and isolated two new C_{29} sterols, colebrin A (**1**) and colebrin B (**2**), together with a known compound, clerosterol (**3**). The present paper deals with the structural elucidation of the new compounds.

Colebrin A (**1**) has a molecular formula of $C_{30}H_{48}O_3$, which was deduced by HRFAB – MS ($[M+1]^+$ 457.3771, calcd. 457.3682) together with ^{13}C-NMR and DEPT spectra. The IR spectrum showed absorption bands (3411 br., 3072, 1722, 1668, 1645 and 887 cm^{-1}) which corresponded to free hydroxyl groups, carboxylic group and olefinic bonds. The presence of these groups were confirmed by ^{13}C NMR spectral data of **1** (**Table 1**). Direct comparison of ^{13}C and ^{1}H NMR data (**Table 1** and **Notes**) of **1** with those of **3** (clerosterol[6]) led to two conclusions: (**a**) **1** had the same carbon skeleton and configuration at C–24 (*i.e* 24 S/β) as **3**; (**b**) one methylene ($\delta_{C\text{-}7}$ 31.90) in **3** was replaced by an oxymethine ($\delta_{C\text{-}7}$ 68.82, $\delta_{H\text{-}7}$ 5.06) in **1** and an additional formyloxy group (δ_C 160.82, δ_H 8.04) appeared in **1**. From the HMQC, HMBC and ^{1}H-^{1}H COSY spectra of **1** (**Table 2**), the new oxymethine was readily assigned to C-7 position. Meanwhile, two pairs of significant ^{1}H-^{13}C long range correlations between H-7 with formyloxy carbon, and formyloxy hydrogen with C-7 could be clearly observed. Thus, it was confirmed that the formyloxy group was attached at C-7 position. Further examining the ^{1}H NMR spectrum of **1** led to the result that the formyloxy group was in α-orientation. On the basis of the HMQC spectrum, the H-6 and H-7 were determined as δ5.56 (1H, d, J = 5.3

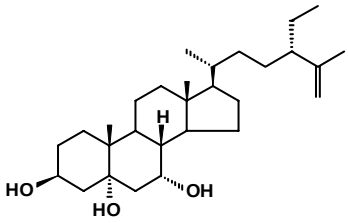
Hz) and 5.06 (1H, t, *J* = 5.3 Hz), respectively. Thus the H-6 coupled with H-7β splitting into a doublet due to a certain dihedral angle, and H-7β coupled with H-6 and H-8β splitting into a triplet owing to two equal dihedral angles. On the contrary, if the formyloxy group were in β-orientation, H-6 and H-7α should be a singlet and a doublet, respectively. In this case, H-6 did not couple with H-7α due to a *ca.* 90° dihedral angle and H-7α coupled only with H-8β. All these observations indicated that the formyloxy group was in α-orientation. The conclusion was also supported by comparing the ^{1}H NMR spectral data of **1** with those of 24-methylene-5-cholesten-3β, 7α-diol and 24-methylene-5-cholesten-3β, 7β-diol[9]. Consequently, the structure of colebrin A was established to be 7 α -formyloxy-clerosterol (**1**). Its structure was shown in **Figure 1**.

Figure 1



1 R = OCH(=O)

3 R = H



2

Table 1 ^{13}C NMR spectral data of compound **1**, **2** and **3** in $CDCl_3$(100.6 MHz, δ in ppm from TMS)

C	1	2	3	C	1	2	3
1	36.73 t	37.46 t	37.23 t	**16**	29.35 t	29.34 t	29.39 t
2	31.20 t	31.92 t	31.62 t	**17**	55.82 d	56.27 d	56.05 d
3	71.11 d	67.59 d	71.76 d	**18**	11.40 q	11.60 q	11.81 q
4	41.89 t	35.79 t	42.28 t	**19**	18.63 q	18.67 q	19.35 q
5	148.58 s	88.80 s	140.68 s	**20**	35.79 d	33.55 d	35.50 d
6	119.4 0d	34.55 t	121.66 d	**21**	18.15 q	17.94 q	18.62 q
7	68.82 d	68.39 d	31.88 t	**22**	33.63 t	33.70 t	33.65 t
8	35.43 d	30.29 d	31.88 d	**23**	23.92 t	22.67 t	24.25 t
9	43.10 d	45.79 d	50.12 d	**24**	50.02 d	49.53 d	49.49 d
10	37.25 s	39.62 s	36.47 s	**25**	147.49 s	147.56 s	147.48 s
11	20.66 t	21.24 t	21.06 t	**26**	111.28 t	111.29 t	111.30 t
12	39.03 t	40.13 t	39.76 t	**27**	17.85 q	17.30 q	17.79 q
13	42.22 s	42.83 s	42.28 s	**28**	26.46 t	26.54 t	26.48 t
14	49.47 d	56.27 d	56.75 d	**29**	11.93 q	12.00 q	12.01 q
15	28.16 t	28.14 t	28.12 t	**Formyl-oxy**	160.82 d		

Colebrin B (**2**) contained five methyl groups (including one primary methyl group, one secondary methyl group and three tertiary methyl groups), twelve methylenes (including one terminal methylene), eight methines (including two oxymethines) and four quaternary carbons judging from its ^{1}H and ^{13}C NMR spectra (**Table 1** and **Notes**).

These facts, together with HRFAB–MS ([M-H_2O+1]$^+$429.3696, calcd. 429.3733), showed that **2** had a molecular formula of $C_{29}H_{50}O_3$. Analysis of ^1H and ^{13}C NMR spectral data revealed that **2** closely resembled clerosterol (**3**)[6]. The differences between **2** and **3** were that (**a**): the carbon signals at δ140.68 (C-5) and 121.66 (C-6) ppm arising from $\Delta^{5,6}$-double bond in **3** were replaced by those at δ88.80 (C-5) and 34.55 (C-6) ppm assignable to a quaternary carbon bearing oxygen and a methylene in **2**, respectively; (**b**): the carbon signal at δ31.90 ppm (C-7) corresponding to a methylene in **3** appeared instead of that at δ68.39 ppm (C-7) due to an oxymethine in **2**. It implied that compound **2** possessed two more hydroxyl groups than **3**, which was also confirmed by its FABMS and IR spectra (their data were listed in **Notes**). On the basis of 2D NMR spectral analysis (including HMQC, HMBC and ^1H-^1H COSY) (**Table 3**), the two additional hydroxyl groups were connected to C-5 and C-7, respectively. Therefore, compound **2** was identified as (24S/β)-stigmasta- 25-en-3β, 5α, 7α-triol. The configurations at C-3, C-5 and C-7 of **2** were confirmed by comparison with relevant compounds[6, 10-12].

Table 2 Some principal results from HMQC, ^1H-^1H COSY and HMBC correlations of **1**

Proton	HMQC(^{13}C)	COSY(^1H)	HMBC(^{13}C)
3α	3	2, 4	5
6	6	7β	4, (7), 8, 10
7β	7	6, 8	5,(6),(8),9, formyloxy-C
18	18		12, (13), 14, 17
19	19		1, 5, 9, (10)
21	21	20	17, (20), 22
26	26	26	24, (25), 27
27	27		24, (25), 26
29	29	28	24, (28)
formyloxy-H	formyloxy-C		7

Two-bond correlations were shown in brackets.

Table 3 Some principal results from HMQC, ^1H-^1H COSY and HMBC correlations of **2**

	HMQC(^{13}C)	COSY(^1H)	HMBC(^{13}C)
3α	3	2, 4	5
4	4	3	2, (3), (5), 10
7β	7	6, 8	5, (8), 9, 14
18	18		12, (13), 14, 17
19	19		1, 5, 9, (10)
21	21	20	17, (20), 22
26	26	26	24, (25), 27
27	27		24, (25), 26
29	29	28	24, (28)

Two-bond correlations were shown in brackets.

Acknowledgment

The authors are grateful for financial support from the National Natural Science Foundation of China (29772039).

References and Notes

1. Yunnan Medicinal Material Corporation, *Resource Lists of Yunnan Traditional Chinese Medicine*, Science Press, Beijing, **1993**, 451.
2. K.C. Joshi, P. Singh, A. Mehra, *Planta Medica*, **1979**, *37* (1), 64.
3. M. Singh, P. K. Chauduri, R.P. Sharma, *et al*, *Indian J. Chem., Sect. B: Org. Chem. Ind. Med. Chem.Soc.*, **1995**, *34B* (8), 753.
4. P. Goswami, J. Kotoky, *J. Indian Chem. Soc.*, **1995**, *72* (9), 647.
5. T. N. Misra, R. S. Sghin, H. S. Pandey, *et al*, *Fitoterapis*, **1995**, *66* (6), 555.
6. P. Goswami, J. Kotok, Z.N. Chen, Y. Lu, *Phytochemistry*, **1996**, *41* (1), 279.
7. T. N. Misra, *et al*, *Indian J.Chem. Sect. B: Org. Chem. Ind. Med. Chem.*, **1997**, *36B* (2), 203.
8. H.Yang, B.Jiang, Z.W. Lin, H.D.Sun, *Journal of Asia Natural Product Research* (in press).
9. J.A. Findoay, A.D. Patil, *Can. J. Chem.*, **1985**, *63*, 2406.
10. M. Kuwabara,, *et al*, *J. Lipid Res.*, **1984**, *25*, 361.
11. B. Dayal, *et al*, *J. Lipid Res.* **, 1985,** *26*, 298.
12. Y.Noma, *et al*, *Chem. Pharm. Bull.*, **1976**, *24*, 2686.
13. Colebrin A (**1**): $C_{30}H_{48}O_3$, colorless wax, $[\alpha]_D^{20.5}$-117 (c 0.112, MeOH); IRν cm^{-1}: 3411 (br.), 3072, 2935, 2870, 1722, 1668, 1645, 1461, 1377, 1179, 1059, 951, 887; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.56 (1H, m, H-3α), 5.56 (1H, d, *J* = 5.3Hz, H-6), 5.06 (1H, t, *J* = 5.3 Hz, H-7β), 0.65 (3H, s, H-18), 0.98 (3H, s, H-19), 0.88 (3H, d, *J* = 6.5 Hz, H-21), 4.70 (1H, br. d, *J* = 2.5 Hz, H-26a), 4.61 (1H, br.d, *J* = 2.5 Hz, H-26b), 1.54 (3H, s, H-27), 0.77 (3H, t, *J* = 7.4 Hz, H-29), 8.04 (1H, s, formyloxy); positive ion FABMS (MNBA) *m/z*: 457 $[M+1]^+$ (8), 439 $[457-H_2O]^+$ (6), 411 (81), 393 (63), 255 (14), 159 (38), 145 (28).
14. Colebrin B (**2**): $C_{29}H_{50}O_3$, colorless wax, $[\alpha]_D^{20.5}$ 0 (c 0.212, MeOH); IRν cm^{-1}: 3432 (br.), 2928, 2855, 1460, 1377, 1271, 890; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.65 (1H, m, H-3α), 2.88 (2H, dd, *J* = 12.1, 5.1 Hz, H-4), 4.64 (1H, br.s, H-7α), 0.65 (3H, s, H-18), 1.18 (3H, s, H-19), 0.88 (3H, d, *J* = 6.4 Hz, H-21), 4.70 (1H, br.d, *J* = 2.3 Hz, H-26a), 4.61 (1H, br.d, *J* = 2.3 Hz, H-26b), 1.54 (3H, s, H-27), 0.77 (3H, t, *J* = 7.4 Hz, H-29); positive ion FABMS (MNBA) *m/z*: 429 $[M-H_2O+1]^+$(5), 411 $[M-2H_2O+1]^+$ (22), 393 (7), 255 (6), 159 (8), 145 (11).
15. Clerosterol (**3**): $C_{29}H_{48}O$, white powder, mp: 119.5~121 °C, $[\alpha]_D^{25.2}$ - 40.87 (c 0.470, $CHCl_3$); IRν^{KBr} cm^{-1}: 3310 ~ 3500, 2915, 1625, 1430, 1364, 1048, 878; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.49 (1H, m, H-3α), 5.32 (1H, br.d, *J* = 5.1 Hz, H-6), 0.64 (3H, s, H-18), 0.97 (3H, s, H-19), 0.88 (3H, d, *J* = 6.5 Hz, H-21), 4.70 (1H, br. d, *J* = 2.5 Hz, H-26a), 4.61 (1H, br.d, *J* = 2.5 Hz, H-26b), 1.53 (3H, s, H-27), 0.77 (3H, t, *J* = 7.4 Hz, H-29); EIMS (70 eV) *m/z*: 412 $[M]^+$ (94), 397 $[M-Me]^+$ (45), 394 $[M-H_2O]^+$ (38), 379 $[M-Me-H_2O]^+$ (35), 328 (40), 314 (61), 300 (50), 299 (62), 271 (73), 255 (44), 159 (57), 145 (64), 119 (62).

Received 29 July 1999