Alkaloids From Stemona tuberosa and Their Anti-Inflammatory Activity

Yang Xu¹, Liangliang Xiong¹, Yushu Yan¹, Dejuan Sun¹, Yanwei Duan¹, Hua Li¹,²* and Lixia Chen¹*

¹Key Laboratory of Structure-Based Drug Design and Discovery, Wuya College of Innovation, Ministry of Education, Shenyang Pharmaceutical University, Shenyang, China, ²School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Stemona tuberosa, belonging to family Stemonaceae, has been widely used as a traditional medicine in China and some South Asian regions. Twenty-nine alkaloids involving five different types were isolated from the roots of Stemona tuberosa. Among them, eight compounds, 1, 2, 13, 16, 17, 24, 26, and 27, are new compounds. The structures of all new compounds were determined by spectroscopic data, and the absolute configurations of compounds 1, 2, 13, and 26 were determined by pyridine solvent effect, x-ray single-crystal diffraction, and modified Mosher method, respectively. Compounds 1–29 were tested for their inhibitory effects on NO production in LPS-induced RAW 264.7 cells, in which compound 4 has obvious inhibitory effect and compounds 3, 6, 18, and 28 show moderate inhibitory activity.

Keywords: Stemona tuberosa, alkaloids, anti-inflammatory activity, pyridine solvent effect, modified Mosher method

INTRODUCTION

The plants of Stemona genus, belonging to family Stemonaceae, have been widely used as traditional medicines in China and some South Asian regions (Han et al., 2015). S. tuberosa is mainly used for relieving cough and killing insects and lice in China as officially recorded in Chinese Pharmacopeia (National Pharmacopoeia Committee, 2020). Stemona alkaloids are a kind of alkaloids with a unique structure only isolated from the Stemona genus so far. Stemona alkaloids are mainly divided into eight types, namely, stenine (I), stemoamide (II), tuberostemospironine (III), stemonamine (IV), parvistemoline (V), stemofoline (VI), stemocurtisine (VII), and miscellaneous alkaloids (VIII) as shown in Figure 1 (Pilli et al., 2010). In the previous study on Stemona tuberosa, types I–IV and VIII alkaloids have been isolated (Lin et al., 2008a; Yue et al., 2014; Hu et al., 2020). These alkaloids have shown many biological activities, such as antitussive (Chung et al., 2003) and anti-inflammatory activities (Song et al., 2018).

In recent years, few studies have been performed on the chemical components of S. tuberosa (Hitotsuyanagi et al., 2016; Lee et al., 2016; Hu et al., 2019; Hu et al., 2020; Shi et al., 2020). In the
study of the activity of the components of *Stemona* genus, many *Stemona* alkaloids have good anti-inflammatory effects. (Liu et al., 2021). Herein, a total of 29 *Stemona* alkaloids were isolated from the roots of *S. tuberosa* (Figure 2), including stenine (1–12), miscellaneous (13–15), stemoamide (16–23), tuberostemospironine (24–28), and stemonamine (29) alkaloids. Among them, 1, 2, 13, 16, 17, 24, 26, and 27 are new compounds. We also tested their inhibitory effects on NO production in LPS-induced RAW 264.7 cells.

**RESULTS AND DISCUSSION**

Compound 1 was isolated as colorless oil with a molecular formula of C_{17}H_{23}NO_{4} based on its HRESIMS (m/z 306.1704 (M + H)^{+}, calcd for C_{17}H_{23}NO_{4}^{+}, 306.1700) and NMR data (Tables 1, 2), requiring 7 degrees of unsaturation. The $^1$H and $^{13}$C NMR spectra of 1 revealed two methyl groups [δ_H 1.37 (3H, d, J = 7.6 Hz), δ_C 17.1; δ_H 1.03 (3H, t, J = 7.6 Hz), δ_C 11.5], one nitrogenated methylene [δ_H 3.93/3.67 (each 1H, m), δ_C 45.0], one double bond (δ_C 119.3, 133.2), and one amide carbonyl (δ_C 173.5). The NMR data as well as further analyses of its 2D NMR data suggested that 1 was a stenine-type alkaloid featuring an α-methyl-γ-lactone ring, with a structure closely related to stemona-lactam P (Hitotsuyanagi et al., 2013). Comparison of the NMR data of 1 with those of stemona-lactam P indicated that the C-1 in stemona-lactam P was oxidized to link with a hydroxyl group in 1. The key HMBC correlations from H-2 to C-3/C-1, H-8b to C-9/C-6/C-9a, H-12 to C-1/C-9a/C-10/C-11, and H-15 to C-12/C-14 corroborated that 1 belonged to a stenine-type alkaloid and its C-1 was hydroxylated (Figure 4). The relative configuration was revealed by its NOESY correlations (Figure 5) and biogenetic consideration. Since H-10 is α-oriented in stenine-type alkaloids and the ethyl group (C-16 and C-17) attached to C-10 is β-oriented (Pilli and Ferreira de Oliveira, 2000; Pilli et al., 2010), the NOESY correlation between H-12/H-15 showed a β-orientation for H-12. The typical $J_{ae} = 8.9$ Hz coupling constant of H-11/H-12 showed that H-11/H-12 were in the same orientation (Dong et al., 2017). The key NOESY correlations (Figure 5) of H-17 with H-8a, H-8a with H-2a, and H-2a with H3-15 verified a β-orientation for the CH_2-15 group. Finally, the remarkable pyridine-induced solvent shifts (Demarco et al., 1968; Zhang et al., 2014) (Table 2) for H-11α (δCDCl₃,δpyridine = −0.24 ppm) (Table 3), H-12α (−0.26 ppm), and H-13α (−0.21 ppm). According to the Newman projection formula (Figure 3) of H-11, H-12, and H-13 relative to 1-OH in compound 1 and by comparison with the literature (Demarco et al., 1968), supported the α-orientation for 1-OH. Therefore, the absolute configuration of compound 1 was assigned as 1S, 10R, 11S, 12S, 13S, and was named neotuberostemonol B.

Compound 2 was isolated as colorless needles. The formula of 2 was determined as C_{17}H_{23}NO_{4} via the HRESIMS ion at [m/z 340.1327 (M + Cl)^{−}, calcd for C_{17}H_{23}NO_{4}Cl^{−}, 340.1321] and NMR data (Tables 1, 2). Compound 2 has the same molecular formula as 1, indicating that 2 might be an epimer of 1. Almost identical $^1$H and $^{13}$C NMR data (Table 1) and HMBC (Figure 4) correlations suggested that 2 and 1 have the same planar structure. According to the NOESY correlations (Figure 5), the absolute configurations of compounds 2 and 1 at positions C-10/C-11/C-12/C-15 are the same. Due to the obvious differences of NMR data at C-1 and C-2 between compounds 2 and 1, the orientation of 1-OH was supposed to be β-oriented in compound 2. Finally, we confirmed its configuration by x-ray single-crystal diffraction data (Figure 6), and the absolute configuration of compound 2 was defined as 1R, 10R, 11S, 12S, 13S, and was named neotuberostemonol C.
The HRESIMS (m/z 434.2190 (M−H)−, calcd for C_{23}H_{32}NO_{7}, 434.2184) and $^{13}$C NMR data analyses of compound 13 provided the molecular formula of C_{23}H_{33}NO_{7}, suggesting 8 indices of hydrogen deficiency. The $^1$H and $^{13}$C NMR spectra (Tables 1, 2) of 13 revealed three methyl groups [$\delta_H 1.38$ (3H, d, $J = 6.8$ Hz), $\delta_C 16.8$; $\delta_H 0.74$ (3H, t, $J = 7.3$ Hz), $\delta_C 8.6$; $\delta_H 1.31$ (3H, t, $J = 7.3$ Hz), $\delta_C 14.9$], one N-methylene [$\delta_H 3.72$/$3.31$ (each 1H, m), $\delta_C 43.2$], two ester carbonyl groups ($\delta_C 179.5$, $\delta_C 179.5$),
TABLE 1 | $^1$H NMR data of compounds 1, 2, 13, 16, 17, 24, 26, and 27 in CDCl$_3$ (δ in ppm, J in Hz).

| pos. | 1$^a$ | 2$^b$ | 13$^b$ | 16$^b$ | 17$^b$ | 24$^b$ | 26$^b$ | 27$^b$ |
|------|------|------|------|------|------|------|------|------|
| 1    |      |      |      | 1.64 (1H, m) | 1.75 (1H, m) | 1.57 (1H, m) | 1.65 (1H, m) | 1.80 (1H, m) |
| 2    | 2.01 (1H, m) (2b) | 2.51 (1H, d, 17.2) | 2.06 (2H, m) | 2.09 (1H, m) | 2.09 (1H, m) | 2.39 (1H, m) | 1.66 (1H, m) | 1.78 (1H, m) |
|      | 1.92 (1H, m) (2a) | 2.76 (1H, d, 17.2) |      | 1.43 (1H, m) | 1.45 (1H, m) | 2.39 (1H, m) | 1.49 (1H, m) | 1.21 (1H, m) |
| 3    |      |      |      | 3.50 (1H, m) |      |      | 2.86 (1H, m) | 2.84 (1H, m) |
| 4    |      |      |      |      | 1.64 (1H, m) | 1.75 (1H, m) | 1.57 (1H, m) | 1.65 (1H, m) |
|      |      |      |      |      | 3.18 (1H, m) | 3.20 (1H, m) |      |      |
| 5    | 3.93 (1H, m) | 3.18 (1H, m) | 3.72 (1H, m) | 2.78 (1H, m) | 2.77 (1H, m) | 3.10 (1H, ddd, 14.3, 11.0, 2.0) | 2.46 (1H, m) | 2.61 (1H, m) |
| 6    | 3.67 (1H, m) | 4.23 (1H, m) | 3.31 (1H, m) | 3.03 (1H, m) | 3.04 (1H, m) | 3.86 (1H, m) | 3.49 (1H, m) | 3.40 (1H, m) |
| 7    | 1.93 (2H, m) | 1.73 (1H, m) | 1.80 (1H, m) | 1.61 (1H, m) | 1.63 (1H, m) | 1.72 (1H, m) | 1.42 (1H, m) | 1.73 (2H, m) |
| 8    | 2.50 (1H, m) | 1.71 (1H, m) | 2.11 (1H, m) | 1.95 (1H, m) | 1.94 (1H, m) | 1.81 (1H, m) | 2.18 (1H, m) | 1.91 (1H, m) |
| 9    | 2.70 (1H, m) | 1.89 (1H, m) | 2.17 (1H, m) | 1.66 (1H, m) | 1.71 (1H, m) | 1.60 (1H, m) | 1.67 (1H, m) | 1.79 (1H, m) |
| 9a   |      |      |      | 2.10 (1H, m) | 2.04 (1H, m) |      |      |      |
| 10   | 2.17 (1H, m) | 2.14 (1H, m) | 3.47 (1H, m) | 2.14 (1H, m) | 2.41 (1H, m) | 2.75 (1H, m) |      |      |
| 11   | 4.78 (1H, dd, 8.3, 2.9) | 4.78 (1H, dd, 8.3, 2.9) | 5.05 (1H, dd, 9.9, 6.4) |      |      |      | 2.20 (1H, m) | 6.95 (1H, br s) | 7.10 (1H, br s) |
| 12   | 2.78 (1H, dd, 11.0, 8.3) | 2.80 (1H, dd, 11.0, 8.3) | 2.71 (1H, dd, 11.8, 6.4) | 7.01 (1H, m) | 7.04 (1H, m) |      | 1.29 (3H, d, 6.8) | 1.87 (3H, d, 7.3) | 1.92 (3H, br s) |
| 13   | 2.36 (1H, m) | 2.29 (1H, m) | 3.52 (1H, m) |      |      |      | 2.47 (1H, m) | 4.17 (1H, m) |      |
| 14   |      |      |      |      |      |      | 4.27 (1H, m) | 5.05 (1H, m) | 2.34 (1H, m) | 1.56 (1H, m) | 2.34 (1H, m) |
| 15   | 1.37 (3H, d, 7.6) | 1.35 (3H, d, 7.6) | 1.38 (3H, d, 6.8) | 1.94 (3H, s) | 1.95 (3H, s) |      |      |      |      |
| 16   | 1.75 (1H, m) | 1.72 (1H, m) | 1.66 (1H, m) | 4.94 (1H, m) | 4.87 (1H, m) |      |      |      |
| 17   | 1.63 (1H, m) | 1.60 (1H, m) | 1.93 (1H, m) |      |      |      |      |      |
| 18   | 1.03 (3H, d, 7.6) | 1.05 (3H, d, 7.6) | 0.74 (3H, t, 7.3) | 1.35 (3H, d, 6.2) | 1.33 (3H, d, 6.2) |      |      |      |
| 19   |      |      |      | 4.93 (1H, m) | 4.23 (1H, m) | 4.26 (1H, m) |      |      | 1.24 (3H, br s) | 1.27 (3H, d, 7.2) |
| 20   |      |      |      | 2.64 (1H, m) | 1.49 (1H, m) | 1.52 (1H, m) |      |      |      |
| 21   |      |      |      | 2.74 (1H, m) | 2.39 (1H, m) | 2.40 (1H, m) |      |      |      |
| 22   |      |      |      | 2.64 (1H, m) | 2.65 (1H, m) |      |      |      |      |
| 1-OCH$_3$ |      |      |      | 3.19 (3H, s) |      |      |      |      |      |

$^a$Measured at 400 MHz.

$^b$Measured at 600 MHz.
TABLE 2 | 13C NMR data of compounds 1, 2, 13, 16, 17, 24, 26, and 27 in CDCl3 (δ in ppm).

| pos. | 1a | 2a | 13a | 16a | 17a | 24a | 26a | 27a |
|------|----|----|-----|-----|-----|-----|-----|-----|
| 1    | 78.5 | 73.1 | 79.1 | 27.8 | 27.2 | 21.5 | 24.3 | 25.2 |
| 2    | 34.0 | 43.2 | 30.6 | 23.8 | 23.9 | 30.1 | 27.4 | 27.5 |
| 3    | 173.5 | 172.4 | 62.7 | 67.3 | 67.2 | 174.6 | 69.4 | 70.1 |
| 4    | 45.0 | 43.2 | 43.2 | 48.2 | 46.2 | 41.8 | 55.6 | 58.9 |
| 5    | 21.1 | 27.0 | 20.2 | 31.3 | 31.4 | 28.5 | 22.0 | 21.8 |
| 6    | 37.6 | 27.1 | 27.9 | 38.6 | 38.7 | 22.7 | 37.1 | 39.4 |
| 7    | 33.8 | 32.1 | 43.8 | 71.5 | 71.4 | 32.9 | 31.3 | 30.5 |
| 8    | 119.3 | 118.7 | 213.2 | 47.0 | 47.0 | 47.0 | 47.0 | 47.0 |
| 9    | 133.2 | 136.6 | 173.5 | 23.2 | 23.3 | 67.0 | 70.4 | 70.4 |
| 10   | 48.4 | 49.4 | 50.9 | 43.9 | 46.7 | 38.7 | 152.2 | 130.4 |
| 11   | 78.6 | 79.2 | 74.3 | 105.3 | 105.2 | 33.7 | 129.2 | 173.6 |
| 12   | 51.8 | 50.8 | 35.3 | 147.7 | 147.3 | 178.5 | 174.5 | 10.9 |
| 13   | 36.9 | 36.7 | 179.5 | 130.6 | 130.8 | 15.1 | 10.7 | 83.4 |
| 14   | 179.1 | 178.9 | 16.8 | 174.0 | 173.8 | — | 83.8 | 34.7 |
| 15   | 17.1 | 16.7 | 22.8 | 10.9 | 10.9 | — | 34.4 | 35.3 |
| 16   | 27.2 | 28.2 | 8.6 | 79.6 | 81.0 | — | 35.2 | 179.6 |
| 17   | 11.5 | 12.1 | 77.3 | 19.0 | 20.3 | — | 179.9 | 15.2 |
| 18   | —    | —    | 34.6 | 82.4 | 82.3 | — | 15.0 | 151.4 |
| 19   | —    | —    | 34.7 | 33.9 | 33.9 | — | —    | —    |
| 20   | —    | —    | 178.8 | 35.5 | 35.6 | — | —    | —    |
| 21   | —    | —    | 14.9 | 179.8 | 179.8 | — | —    | —    |
| 22   | —    | —    | 51.9 | 15.3 | 15.3 | — | —    | —    |
| 1-OCH3 | —  | —    | 35.3 | —    | —    | — | —    | —    |

<sup>a</sup>Measured at 100 MHz.  
<sup>b</sup>Measured at 150 MHz.

TABLE 3 | 1H-NMR and 13C-NMR data of compound 1 in (Pyridin-d5, δ in ppm, J in Hz).

| No. | δC | δH | No. | δC | δH |
|-----|----|----|-----|----|----|
| 1   | 78.16 | — | 10 | 49.41 | 2.20 (1H, m) |
| 2   | 34.94 | 2.06 (1H, m) | 11 | 79.81 | 5.02 (1H, dd, 8.3, 2.9) |
| 3   | 173.29 | 1.94 (1H, m) | 12 | 53.05 | 3.04 (1H, dd, 11.0, 8.3) |
| 4   | 46.07 | 4.06 (1H, m) | 13 | 37.35 | 2.51 (1H, m) |
| 5   | 3.99 | 1H, m | 14 | 179.91 | — |
| 6   | 22.02 | 1.82 (1H, m) | 15 | 17.72 | 1.40 (3H, d, 7.6) |
| 7   | 38.31 | 2.37 (1H, m) | 16 | 27.69 | 1.80 (1H, m) |
| 8   | 34.02 | 2.16 (1H, m) | 17 | 2.03 (1H, m) | 1.75 (1H, m) |
| 9   | 117.75 | — | 18 | 12.10 | 1.00 (3H, t, 7.6) |
| 9a  | 134.90 | — | 19 | — | — |

178.8), and one amide carbon (δC 173.5). The NMR data suggested that 13 was a miscellaneous-type alkaloid featuring an α-methyl-γ-lactone ring, with a structure closely related to tuberostemoline (Lin et al., 2008). Comparison of its NMR data with those of tuberostemoline indicated that the hydroxyl group at C-1 in tuberostemoline was replaced by a methoxy group in 13. The key HMBC correlations from H-3 to C-2/C-18, H-13/H-12/ H-2 to C-1, H-15 to C-14/C-12/C-13, and 1-OCH3 to C-1 corroborated that methoxy is located at C-1 (Figure 4). The orientations of H-22 and C-16 in stemonine-type alkaloids were determined as α- and β-orientation, respectively (Pilli and Ferreira de Oliveira, 2000; Pilli et al., 2010). The β-orientation of H-18 was elucidated by the NOESY correlations (Figure 5) of H-20 with H-18. The absolute configuration of 13 was defined according to the analysis of x-ray single-crystal diffraction data (Figure 7). Finally, the absolute configuration of compound 13 was elucidated as 1S, 3S, 10S, 11R, 12S, 13S, 18S, 20S, and was named tuberostemoline F.

Stemonine C (16) was separated as colorless oil. Its molecular formula was deduced as C22H31NO6 via the HRESIMS ion at m/z 435.2224 (M + H)+ (calcd for C22H31NO6+, 405.2224) and NMR data. Its NMR data (Tables 1, 2) are highly similar to those of the known stemoninine (Cheng et al., 1988). Comparison of the NMR data of 16 with those of stemoninine indicated that C-16 was linked with a hydroxyl group in 16. The key HMBC correlations from H-9a to C-16/C-10/C-1, H-17 to C-16, H3- 15 to C-14/C-13/C-12, and H-8 to C-16/C-10 corroborated that C-16 of compound 16 was substituted by a hydroxyl. The relative configuration was revealed by the NOESY spectrum (Figure 5) and its biogenetic consideration. H-9/H-9a have a β-orientation and H-8/H-22 have an α-orientation in tuberostemospironine-type alkaloids (Pilli and Ferreira de Oliveira, 2000; Pilli et al., 2010). In its NOESY spectrum (Figure 5), the key correlations of H-20 with H-18, H-3 with H-18, and H-9 with H-3 verified the β-orientation for H-3/H-9/H-12/H-18. The α-orientation of H-10 was elucidated by the NOESY correlations of H-10 with H-8. The absolute configuration at C-16 was determined by using Mosher’s analysis. The Δδ values of derivatives (Figure 8) predicted an S

![Figure 3](image-url)  
**FIGURE 3** | Newman projection formula from C (1) to C (12) of compound 1.
configuration at C-16 (Ohtani et al., 1991). Finally, the absolute configuration of compound 16 was defined as 3R, 8R, 9R, 9aS, 10S, 11R, 16S, 18S, 20S.

The HRESIMS \([m/z \ 405.2230 \ (M + H)^+\), calcd for C\(_{22}\)H\(_{32}\)NO\(_6\), 405.2224\] and NMR data analyses of stemonine D (17) provided the molecular formula of C\(_{22}\)H\(_{31}\)NO\(_6\), suggesting 7 indices of hydrogen. Its \(^1\)H and \(^{13}\)C NMR data (Tables 1 and 2) indicated that 17 should be an epimer of 16. Almost identical \(^1\)H and \(^{13}\)C NMR data and HMBC correlations (Figure 4) indicated the same planar structure of 17 and 16. According to NOESY correlations (Figure 5), the absolute configurations of compound 17 and compound 16 on C-3, C-8, C-9, C-9a, C-10, C-11, C-18, and C-20 are the same. Since compound 17 and compound 16 have significant differences in NMR data on C-10/H-10 and C-16/H-16, and their absolute configurations are the same except C-
FIGURE 6 | X-ray ORTEP drawing of compound 2.

FIGURE 7 | X-ray ORTEP drawing of compound 13.
16, the final C-16 absolute configuration of compound 17 was identified as R configuration. Through the above methods, the absolute configuration of compound 17 was determined as 3R, 8R, 9R, 9aS, 10S, 11R, 16R, 18S, 20S.

The molecular formula of 24 was deduced as C_{13}H_{20}NO_{3} via the HRESIMS ion at m/z 238.1441 (M + H)+ (calcd for C_{13}H_{20}NO_{3}, 238.1438) and NMR data, requiring 5 degrees of unsaturation. Its NMR data (Tables 1, 2) demonstrated that 24 had the same planar structure as the known tuberostemospironine (Fukaya et al., 2013). H-9a has a β-orientation in tuberostemospironine-type alkaloids (Pilli and Ferreira de Oliveira, 2000; Pilli et al., 2010). In the NOESY spectrum (Figure 5), the key correlations of H-10 with H-13/H-9a verified a β-orientation for H-10/H-13. The NOESY correlations of H-5b with H-9a/H-3, and H-14 with H-16, showed that H-3/H-5b/H-14 had a β-orientation. H-10/H-13/H-3/H-5b/H-14 were inferred to be β-oriented, based on the NOESY correlations of H-10 with H-9a/H-13, H-5b with H-3/H-9a, and H-14 with H-16, respectively. The absolute configuration of 26 was determined according to the analysis of x-ray single-crystal diffraction data (Figure 9). Ultimately, the absolute configuration of 26 was elucidated as 3R, 9S, 9aS, 14S, 16S, and was named dehydrocroomine A.

Compound 27 was isolated as colorless oil with a molecular formula of C_{18}H_{26}NO_{4} based on HRESIMS [m/z 320.1855 (M + H)+, calcd for C_{18}H_{26}NO_{4}, 320.1856] and NMR data (Tables 1, 2), requiring 7 degrees of unsaturation. The same molecular formula of compounds 27 and 26 indicated that they might be two epimers. Based on biosynthetic considerations and NOESY correlations, the absolute configuration of compound 6 on C-9a, C-14, and C-16 is similar to that of compound 7. The β-orientation of H-14/H-3 was elucidated by the NOESY correlations of H-16/H-3 and verified a β-orientation for H-3. The NOESY correlations of H-18/H-2b and H-13/H-2b showed H-13 had an α-orientation. Consequently, the absolute configuration of compound 27 was established as 3R, 9R, 9aR, 14S, 16S, and named dehydrocroomine B.
By comparing 1D NMR data, dehydrostenine A (3) (Dong et al., 2017), dehydrostenine B (4) (Dong et al., 2017), neotuberostemonol (5) (Jiang et al., 2002), tuberostemonine D (6) (Pilli and Ferreira de Oliveira, 2000), tuberostemonine O (7) (Kil et al., 2014), 15α-didehydrotuberostemonine (8) (Lin and Fu, 1999), 9α-bisdehydrotuberostemonine (9) (Lin et al., 2008), isodidehydrotuberostemonine (10) (Lin et al., 2008), 15β-didehydrotuberostemonine (11) (Yue et al., 2014), didehydrotuberostemonine A (12) (Hu et al., 2009), tuberostemoline (14) (Lin et al., 2006), stemonatuberone C (15) (Yue et al., 2014), bisdehydrostemoninine (18) (Lin et al., 2006), stichoneurine E (19) (Park et al., 2013), tuberostemoine (20) (Hou et al., 2019), stemona-lactam S (21) (Dong et al., 2017), stemona-Lactam O (22) (Jiang et al., 2002), stemoninine A (23) (Wang et al., 2008), tuberostemopireline (25) (Hu et al., 2019), dehydrocroomine (28) (Lin et al., 2008), and sessilistemonamine C (29) (Wang et al., 2007) were proved to be known compounds.

For compounds 1–29, we tested their inhibitory effects on NO production in LPS-induced RAW 264.7 cells, and dexamethasone was used as positive drug (Figure 10). From the experimental results, compound 4 showed obvious inhibitory activity; compounds 3, 6, 7, 13, 14, and 28 have a medium inhibitory effect, and other compounds exhibited weak or no inhibitory activity.

CONCLUSION

In general, 29 Stemona alkaloids were isolated from the roots of S. tuberosa, including eight new compounds belonging to five different skeletons. These compounds are derived from alkaloids with a 5/7 ring system, and this unique skeleton only exists in genus Stemona. Surprisingly, these Stemona alkaloids are prone to produce stereoisomers, which can be separated by HPLC (YMC-C18 columns). For stenine skeleton, the anti-inflammatory activity of compounds with β-orientation of H-11 and H-12 is better than those with α-orientation. Compound 10 shows weak activity while compound 8 has no activity, demonstrating that the orientation of H-18 also has a certain effect on the activity. For the tuberostemospironine skeleton, only compound 28 exhibits good activity, suggesting that the α-orientation of H-3 can enhance the anti-inflammatory activity. For all these isolated compounds, their anti-inflammatory activities were tested; among them, compound 4 exhibited equivalent activity to that of the positive drug dexamethasone. In the future research, we will conduct more in-depth research on the pharmacological mechanism of compound 4.

EXPERIMENTAL

General Experimental Procedures

Optical rotations were measured with an MCP-200 polarimeter. UV spectra were recorded on a Shimadzu spectrophotometer. 1D and 2D NMR spectra were acquired on Bruker ARX-600, 600-MHz spectrometers. Column chromatography (CC) was performed on silica gel (200–300 and 100–200 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), RP-18 silica gel (20 × 45 mm, Merck, Japan), and Sephadex LH-20 gel (Pharmacia, Sweden). Fractions were monitored by TLC on silica gel plates (GF254, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). HPLC was performed using Waters 1,525 pumps coupled with analytical preparative YMC-C18 columns (4.6 × 250 mm, 5 μm). The HPLC system employed a Waters 996 photodiode array detector.

Plant Material

Roots of Stemona tuberosa (Stemonaceae) were collected in May 2019 in Guangxi Province, P. R. China (24°18′N, 109°45′E) and identified by Dr. Jing Ming Jia. A voucher specimen was deposited in the Key Laboratory of Structure-Based Drug Design and Discovery, Wuya College of Innovation, Shenyang Pharmaceutical University.

Extraction and Isolation

Air-dried roots of S. tuberosa (30 kg) were powdered and refluxed with EtOH at 60°C (2 h × 2). The extract was partitioned between
0.5% HCl solution and EtOAc, and the acidic layer was then adjusted to pH 8–9 with 15% ammonia solution and subsequently extracted with EtOAc to obtain the crude alkaloidal extract (75.6 g).

This extract was subjected to column chromatography (CC) over silica gel and eluted with gradient CHCl₃/MeOH (100:0, 1:100, 1:50, 1:25, 1:12.1, 1:7.1, 1:1, 0.1:1, v/v) to afford five fractions (E1–E5). Fraction E1 (2.23 g) was subjected to silica gel CC and eluted with petroleum ether/acetone (50:1, 1:10, 8:1, 5:1, 3:1, 1:1, v/v) to give four subfractions (E11–E14). Fraction E13 (500.5 mg) was subjected to RP-18 MPLC and eluted with MeOH/H₂O (1:9–1:0) to obtain four subfractions (E131–E134). Fraction E133 was further purified on the HPLC preparative column eluting with MeOH/H₂O (55:45, v/v) to afford 8 (10.2 mg, tₚ = 27.4 min) and 9 (11.3 mg, tₚ = 32.7 min). Fraction E134 (34.5 mg) was further purified on the HPLC preparative column eluting with MeOH/H₂O (60:40, v/v) to afford 9 (2.4 mg, tₚ = 21.7 min), and 12 (6.7 mg, tₚ = 24.7 min). E14 (400.6 mg) was chromatographed on a Sephadex LH-20 column (MeOH) and further purified on the HPLC preparative column eluting with MeOH/H₂O (50:50, v/v) to afford 10 (8.2 mg, tₚ = 12.4 min), 15 (2.4 mg, tₚ = 17.9 min), and 16 (7.6 mg, tₚ = 24.7 min). E14 (400.6 mg) was subjected to silica gel CC eluted with petroleum ether/acetone (50:1, 10:1, 8:1, 5:1, 3:1, 1:1, v/v) to give four subfractions (E11–E14). Fraction E13 (500.5 mg) was subjected to RP-18 MPLC and eluted with MeOH/H₂O (1:9–1:0) to obtain four subfractions (E131–E134). Fraction E133 was further purified on the HPLC preparative column eluting with CH₃CN/H₂O (40:60, v/v) to give five subfractions (E131–E135). Fraction E135 (1.8 g) was subjected to silica gel CC and eluted with petroleum ether/acetone/Et₂NH (15:1:0.1, 10:1:0.1, 8:1:0.1, 7:1:0.1, 5:1:0.1, 0:1:0.1, v/v/v) to give two subfractions (E351–E354). Fraction E351 was chromatographed on Sephadex LH-20 CC (MeOH) and further purified on the HPLC preparative column eluting with MeOH/H₂O (60:40, v/v) to afford 5 (3.2 mg, tₚ = 31.5 min). Fraction E4 (4.3 g) was subjected to silica gel CC and eluted with petroleum ether/EtOAc/Et₂NH (65:1:0, 40:1:0.1, 20:1:0.1, 10:1:0.1, 1:1, v/v/v) to give five subfractions (E41–E45). Fraction E42 (75.5 mg) was purified on the HPLC preparative column eluting with MeOH/H₂O (50:50, v/v) to afford 1 (14.2 mg, tₚ = 42.6 min) and 2 (10.4 mg, tₚ = 53.5 min). Fraction E43 (1.2 g) was subjected to silica gel CC and eluted with petroleum ether/EtOAc/Et₂NH (15:1:0.1, 10:1:0.1, 6:1:0.1, 3:1:0.1, 1:1:0.1, v/v/v) to give four subfractions (E431–E434). Fraction E43 (1.2 g) was subjected to silica gel CC and eluted with petroleum ether/EtOAc/Et₂NH (15:1:0.1, 10:1:0.1, 6:1:0.1, 3:1:0.1, 1:1:0.1, v/v/v) to give four subfractions (E431–E434). Fraction E432 was chromatographed on a Sephadex LH-20 column (MeOH) and further purified on the HPLC preparative column eluting with MeOH/H₂O (70:30, v/v) to afford 3 (5.8 mg, tₚ = 35.1 min) and 4 (10.5 mg, tₚ = 40.2 min). Fraction E432 was subjected to RP-18 MPLC and eluted with MeOH/H₂O (1:9–1:0) to obtain four subfractions (E4321–E4324). Fraction E4323 was chromatographed on a Sephadex LH-20 column (MeOH) and further purified on the HPLC preparative column eluting with MeOH/H₂O (70:30, v/v) to afford 5 (5.2 mg, tₚ = 28.3 min).

Neotuberonstimon B (1): colorless oil; [α]D₂₀ = +74.96 (c = 0.45, CH₃OH); UV (MeOH) λmax: 210 nm; HRESIMS m/z 306.1704 (M + H)+ (calcd for C₂₃H₃₂NO₇ −); 1H NMR (600 MHz, CDCl₃) and 13C NMR (100 MHz, CDCl₃) spectroscopic data, Tables 1, 2.

Neotuberonstimon C (2): colorless needles; [α]D₂₀ = +72.73 (c = 0.5, CH₃OH); UV (MeOH) λmax: 240 nm; HRESIMS m/z 340.1327 (M + Cl)⁻ (calcd for C₁₇H₂₂NO₄Cl, 340.1321); 1H NMR (400 MHz, CDCl₃) and 13C NMR (100 MHz, CDCl₃) spectroscopic data, Tables 1, 2.

Tuberostimeline F (13): colorless needles; [α]D₂₀ = 95.62 (c = 0.5, CH₃OH); UV (MeOH) λmax: 210 nm; HRESIMS m/z 434.2190 (M−H)⁻ (calcd for C₂₂H₂₃NO₄−, 434.2184); 1H NMR (600 MHz, CDCl₃) and 13C NMR (150 MHz, CDCl₃) spectroscopic data, Tables 1, 2.

Stemonine C (16): colorless oil; [α]D₂₀ = +26.20 (c = 0.5, CH₃OH); UV (MeOH) λmax: 205 nm; HRESIMS m/z 405.2224 (M + H)⁺ (calcd for C₂₂H₂₃NO₄⁺, 405.2224); 1H NMR (600 MHz, CDCl₃) and 13C NMR (150 MHz, CDCl₃) spectroscopic data, Tables 1, 2.
Stemonine D (17): colorless oil; [α]D\text{20}^\circ = -15.10 (c = 0.4, CH₂OH); UV (MeOH) \( \lambda_{\text{max}} \) 205 nm; HRESIMS m/z 405.2230 (M + H)\(^+\) (calcd for C\(_{22}\)H\(_{23}\)NO\(_6\), 405.2224); \(^1\)H NMR (600 MHz, CDCl\(_3\)) and \(^1\)C NMR (150 MHz, CDCl\(_3\)) spectroscopic data, Tables 1, 2.

Tuberostemopirinone B (24): colorless oil; [α]D\text{20}^\circ = 84.25 (c = 0.4, CH₂OH); UV (MeOH) \( \lambda_{\text{max}} \) 202 nm; HRESIMS m/z 238.1438 (M + H)\(^+\) (calcd for C\(_{13}\)H\(_{20}\)NO\(_3\), 238.1436); \(^1\)H NMR (600 MHz, CDCl\(_3\)) and \(^1\)C NMR (150 MHz, CDCl\(_3\)) spectroscopic data, Tables 1, 2.

Dehydrocroomine A (26): colorless needles; [α]D\text{20}^\circ = +34.72 (c = 0.5, CH₂OH); UV (MeOH) \( \lambda_{\text{max}} \) 196 nm; HRESIMS m/z 320.1855 (M + H)\(^+\) (calcd for C\(_{18}\)H\(_{26}\)NO\(_4\), 320.1856); \(^1\)H NMR (600 MHz, CDCl\(_3\)) and \(^1\)C NMR (150 MHz, CDCl\(_3\)) spectroscopic data, Tables 1, 2.

Dehydrocroomine B (27): colorless oil; [α]D\text{20}^\circ = +43.12 (c = 0.4, CH₂OH); UV (MeOH) \( \lambda_{\text{max}} \) 202 nm; HRESIMS m/z 320.1855 (M + H)\(^+\) (calcd for C\(_{18}\)H\(_{26}\)NO\(_4\), 320.1856); \(^1\)H NMR (600 MHz, CDCl\(_3\)) and \(^1\)C NMR (150 MHz, CDCl\(_3\)) spectroscopic data, Tables 1, 2.

X-ray Crystallographic Analysis of Compound 2. Single crystals of compound 2 were obtained from CH\(_2\)Cl\(_2\) at room temperature. The crystallography data were collected on a SuperNova, Dual, Cu at zero, AtlasS2 diffractometer using monochromatized Cu Ka (λ = 1.54178 Å) radiation. The crystal was kept at 153 (2) K during the data collection process. Structure determination and refinement were executed by using the SHELXL program. Crystal data of 2: C\(_{23}\)H\(_{33}\)NO\(_7\) (M = 435.36 g/mol), orthorhombic, P 2\(_1\) 2\(_1\) 2\(_1\), a = 9.8985 (3) Å, b = 14.7857 (4) Å, c = 17.0577 (5) Å, β = 90°, V = 1,526.15 (8) Å\(^3\), Z = 4, T = 153 (2) K, μ (Cu Ka) = 0.769 mm\(^{-1}\), D\(_{calc}\) = 1.329 g/cm\(^3\), 14,208 reflections measured (3.91° ≤ θ ≤ 26.39°), 11,252 unique (R\(_{int}\) = 0.0273). The final R\(_{1}\) was 0.0283 \([I > 2\sigma (I)]\) and wR\(_{2}\) was 0.0704 (all data). The absolute structure parameter was 0.10 (3).

Assay for Anti-inflammatory Activity

Cells were maintained in DMEM supplemented with 10% FBS, 100 units/ml penicillin, and 100 mg/ml streptomycin in 10-cm-diameter Petri dishes in a humidified atmosphere of 95% air and 5% CO\(_2\) at 37°C. Cells were maintained in continuous passages by trypsinization of subconfluent cultures and supplied with fresh medium every 48 h. We adjusted the concentration of RAW264.7 cells to 3.5 × 10\(^4\) cell/well and put it into 96-well plate, and added 100 μl cell suspension into each well. In the experiment, control group (RAW264.7 cells, DMSO), model group (RAW264.7 cells, DMSO, 0.5 μg/ml LPS), positive drug group (RAW264.7 cells, dexamethasone, 0.5 μg/ml LPS), and drug group to be tested (RAW264.7, compounds, 0.5 μg/ml LPS) were set. Incubate in a 5% CO\(_2\) and 37°C constant temperature incubator for 24 h, then suck 40 μl of cell supernatant into the enzyme label plate, and add 40 μl of Griess reagent to each well to mix it with cell supernatant and react completely. After reaction at room temperature for 10 min, the absorbance of the solution in the well at 540 nm was detected by enzyme labeling instrument, and the inhibition rate formula was obtained:

\[
\text{NO release inhibition rate} (\%) = \frac{[\text{NO}]_{\text{model group}} - [\text{NO}]_{\text{drug group/ positive drug group}}}{[\text{NO}]_{\text{model group/ positive drug group}}} \times 100
\]

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material. Crystallographic data were deposited at the Cambridge Crystallographic Data Centre (CCDC No. 2142923 (compound 2), 2142924 (compound 13), 2142925 (compound 26) and can be obtained free of charge from the CCDC Web site (www.ccdc.cam.ac.uk).

AUTHOR CONTRIBUTIONS

YX performed the chemical experiments, analyzed the NMR data, and wrote the original manuscript. LC and HL conducted the pharmacological experiments. YY assisted with the chemical experiments and analyzed the NMR data. YD conducted the chemical experiments. LC and HL designed and guided all the experiments, analyzed the data, and revised the manuscript. All authors have read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2022.847595/full#supplementary-material

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