# Dibromotyrosine derivatives from the ethanol extract of the marine sponge *Aplysina* sp.: structures, transformations, and origin

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Twenty nine 3,5-dibromotyrosine derivatives were isolated from the ethanol extract of the marine sponge *Aplysina* sp. (South China Sea) including the earlier unknown compounds, in particular, *p*-hydroxycyclohexadienone and *p*-hydroxycyclohexenone ketals. The isolated enones, dienones, and ketals can be transformation products of aeroplysinin-1 in the course of its reactions with water and alcohols.

**Key words:** marine sponges, dibromotyrosines, alkaloids, aeroplysinin-1, ketals, cyclohexadienones.

Representatives of sponges of the genus *Aplysina* belong to a small group of marine invertebrates possessing mechanism of "activated chemical defense".[1] Thus, when the tissues of *Aplysina* sponge are injured, its deterrent isoxazoline alkaloids are enzymatically cleaved to the more low-molecular-weight antibacterial derivatives with the skeleton of 3,5-dibromotyrosine, that can protect the sponge organism from penetration of pathogenic bacteria.[2] Aeroplysinin-1 (**1**) is the most familiar among dibromotyrosines formed and attracts attention as a potential medicine.[3]

The concept of existence of the mechanism of activated chemical defense in sponges *Aplysina* sp. appeared comparatively recently. Chemistry of the formation of many dibromotyrosine derivatives (enones, dienones, ketals), which together with compound **1** are found in the extracts of *Aplysina*, remains poorly studied. In particular, some authors still suggest the presence of hypothetical arenoxide or imino ether precursors in sponges,[4,5] from which all the spectrum of these simple dibromotyrosines is formed. The insufficient knowledge of chemical processes leading from compounds-precursors to numerous products of their transformation prompted us to carry out detailed structural studies of dibromotyrosine derivatives of the sponge *Aplysina* sp. (the order *Verongida*, the family Aplysinidae; South China Sea) and demonstrate a key role of aeroplysinin-1 (**1**) in the sequence of their transformations suggested on the basis of mechanism of activated chemical defense.

## Results and Discussion

The already described dibromotyrosine derivatives **1**—**15** and new compounds of this group **16**—**29** were isolated from the ethanol extract of the sponge *Aplysina* sp. by column chromatography on Sephadex LH-20 and silica gel with subsequent purification by the reversed-phase HPLC. The earlier described compounds were identified by comparison of their spectral data and physical constants with those given in the literature.[4,6—13] The structures of compounds **16**—**29** were established by $^1$H and $^{13}$C NMR spectroscopy (including the COSY, HMBC, and NOESY experiments), IR spectroscopy, ESI and EI mass spectrometry, as well as quantum chemical calculations. Based on the common origin with (+)-aeroplysinin-1 (**1**),[6] the same absolute configuration of the asymmetric centers C(1) and C(6) was assigned to the rest of the found by us chiral compounds.

***p*-Hydroxycyclohexadienone ketals.** Analysis of the $^1$H NMR spectra (Table 1), IR spectra, and EI mass spectra of configurational isomers **13** and **14** shows that both compounds are the methyl ethyl ketals of *p*-hydroxycyclohexadienone **8**. The NOESY spectrum of 2-(*E*-3,5-dibromo-4-ethoxy-1-hydroxy-4-methoxycyclohexa-2,5-dien-1-yl)acetamide (**13**) exhibits a cross-peak between the $H_2C(7)$ at $\delta_H$ 2.51 (s) and $—OC\underline{H}_2CH_3$ at $\delta_H$ 3.27 (q), that indicates the *cis*-orientation of these group with respect to the plane of the double bond of the ring. Conversely, the NOESY spectrum of 2-(*Z*-3,5-dibromo-4-ethoxy-1-hydroxy-4-methoxycyclohexa-2,5-dien-1-yl)-acetamide (**14**) exhibits cross-peaks between the $H_2C(7)$ at $\delta_H$ 2.51 (s) and $—OCH_3$ at $\delta_H$ 3.16 (s), as well as between the —OH at $\delta_H$ 5.17 (br.s) and $—OC\underline{H}_2CH_3$ at $\delta_H$ 3.36 (q). Comparison of the $^1$H NMR spectra of isomers **13** and **14** shows that the main difference between them consists in position of the signals for the protons of the ether groups at the C(4) atom. Thus, the *E*-isomer **13** has the difference in the chemical shift (CS) values $\Delta\delta_H$ of the

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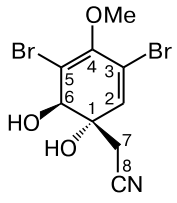

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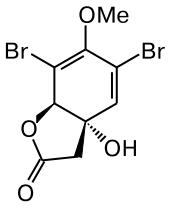

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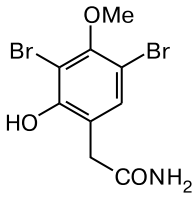

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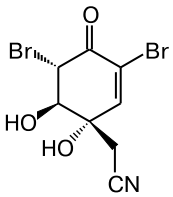

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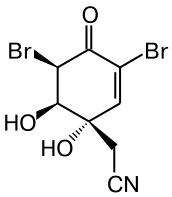

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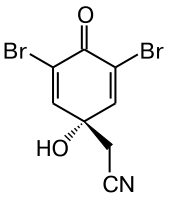

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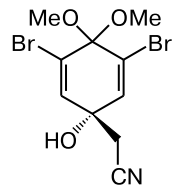

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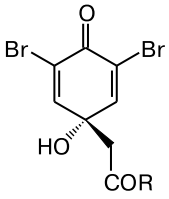

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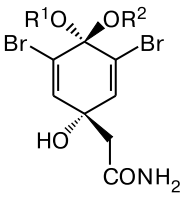

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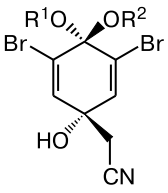

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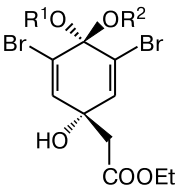

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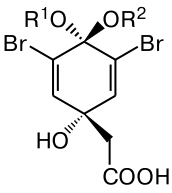

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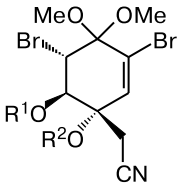

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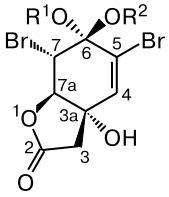

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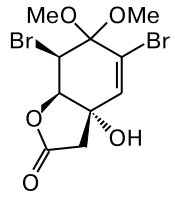

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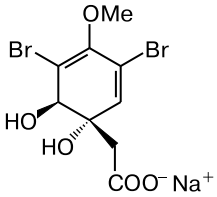

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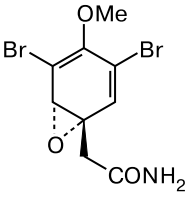

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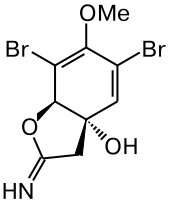


**31**

| Compound | R |
|---|---|
| **8** | $NH_2$ |
| **9** | OH |
| **10** | OMe |
| **11** | OEt |

| Compound | $R^1$ | $R^2$ |
|---|---|---|
| **12** | Me | Me |
| **13** | Me | Et |
| **14** | Et | Me |
| **15** | Bu | Me |
| **16** | Me | Bu |
| **17** | Et | Et |

| Compound | $R^1$ | $R^2$ |
|---|---|---|
| **18** | Me | Et |
| **19** | Et | Me |
| **20** | Me | Et |
| **21** | Et | Me |
| **22** | Et | Et |
| **23** | Me | Et |

| Compound | $R^1$ | $R^2$ |
|---|---|---|
| **24** | Et | Me |
| **25a** | H | H |
| **25b** | Ac | H |
| **25c** | Ac | Ac |
| **26** | Me | Me |
| **27** | Me | Et |

signals for the protons $—OCH_2—$ and $—OCH_3$ equal to 0.06 ppm, whereas the corresponding value for *Z*-isomer **14** is equal to 0.20 ppm. Judging from the $\Delta\delta_H$ value, the latter isomer has been described earlier[9] without assignment of configuration, but with indication on the presence of admixture of a diastereoisomer in it.

Aplysinketal A (**15**)[10] and its earlier unknown isomer 2-(*E*-3,5-dibromo-4-butoxy-1-hydroxy-4-methoxycyclohexa-2,5-dien-1-yl)acetamide (**16**) have close $^1H$ NMR spectra (see Table 1), IR spectra, and EI mass spectra. However, the NOESY spectrum of compound **15** contains a cross-peak between the $H_2C(7)$ ($\delta_H$ 2.51, s) and $—OCH_3$ ($\delta_H$ 3.16, s), whereas the NOESY spectrum of compound **16** has a cross-peak between the $H_2C(7)$ ($\delta_H$ 2.51, s) and $—OC\underline{H}_2CH_2CH_2CH_3$ ($\delta_H$ 3.18, t).

Molecular formula $C_{12}H_{17}Br_2NO_4$ for 2-(3,5-dibromo-4,4-diethoxy-1-hydroxycyclohexa-2,5-dien-1-yl)-acetamide (**17**) was found based on the $^{13}C$ NMR spectroscopic and (+)-ESI mass spectrometric data. The $^1H$ and $^{13}C$ NMR spectra (Tables 1, 2) exhibit signals for the two ethoxy groups at the C(4) atom, whereas the rest of the NMR spectra of homologous ketals **13** and **17** are identical.

According to the $^{13}C$ NMR spectroscopic and (+)-ESI mass spectrometric data, the mixed ketals **18** and **19** containing an acetonitrile side moiety instead of the acetamide one have molecular formulas $C_{11}H_{13}Br_2NO_3$. $^1H$ and $^{13}C$ NMR spectra (see Tables 1 and 2) of these compounds are close to the corresponding spectra of dimethyl ketal **7**,[4] however, the signals for the methoxy and ethoxy groups are present instead of signals for the two methoxy ones at the C(4) atom. The NOESY spectrum of (*E*-3,5-dibromo-4-ethoxy-1-hydroxy-4-methoxycyclohexa-2,5-dien-1-yl)acetonitrile (**18**) exhibits a cross-peak between $H_2C(7)$ ($\delta_H$ 2.73, s) and $—OC\underline{H}_2CH_3$ ($\delta_H$ 3.33, q). The NOESY spectrum of its *Z*-isomer (**19**) shows correlations between the $H_2C(7)$ ($\delta_H$ 2.73, s) and $—OCH_3$ ($\delta_H$ 3.21, s), as well as between the —OH ($\delta_H$ 2.37, br.s) and $—OC\underline{H}_2CH_3$ ($\delta_H$ 3.35, q).

**Table 1.** $^1$H NMR spectra of compounds **13—29***

| Compound | $^1$H NMR, δ (*J*/Hz) |
|---|---|
| **13** | 1.28 (t, 3 H, C(4)OCH$_2$C<u>H</u>$_3$, *J* = 6.7); 2.51 (s, 2 H, C(7)H$_2$); 3.21 (s, 3 H, C(4)OMe); 3.27 (q, 2 H, C(4)OC<u>H</u>$_2$CH$_3$, *J* = 6.7); 5.10 (br.s, 1 H, C(1)OH); 5.59, 5.76 (both br.s, 1 H each, O=C(8)NH$_2$); 6.73 (s, 2 H, C(2)H, C(6)H) |
| **14** | 1.275 (t, 3 H, C(4)OCH$_2$C<u>H</u>$_3$, *J* = 6.8); 2.51 (s, 2 H, C(7)H$_2$); 3.16 (s, 3 H, C(4)OMe); 3.36 (q, 2 H, C(4)OC<u>H</u>$_2$CH$_3$, *J* = 6.8); 5.17 (br.s, 1 H, C(1)OH); 5.67, 5.825 (both br.s, 1 H each, O=C(8)NH$_2$); 6.73 (s, 2 H, C(2)H, C(6)H) |
| **15** | 0.91 (t, 3 H, C(4)OCH$_2$CH$_2$CH$_2$C<u>H</u>$_3$, *J* = 7.3); 1.45 (m, 2 H, C(4)OCH$_2$CH$_2$C<u>H</u>$_2$CH$_3$); 1.62 (m, 2 H, C(4)OCH$_2$C<u>H</u>$_2$CH$_2$CH$_3$); 2.51 (s, 2 H, C(7)H$_2$); 3.16 (s, 3 H, C(4)OMe); 3.28 (t, 2 H, C(4)OC<u>H</u>$_2$CH$_2$CH$_2$CH$_3$, *J* = 6.2); 5.17 (br.s, 1 H, C(1)OH); 5.56, 5.73 (both br.s, 1 H each, O=C(8)NH$_2$); 6.73 (s, 2 H, C(2)H, C(6)H) |
| **16** | 0.92 (t, 3 H, C(4)O(CH$_2$)$_3$C<u>H</u>$_3$, *J* = 7.2); 1.46 (m, 2 H, C(4)O(CH$_2$)$_2$C<u>H</u>$_2$CH$_3$); 1.62 (m, 2 H, C(4)OCH$_2$C<u>H</u>$_2$CH$_2$CH$_3$); 2.51 (s, 2 H, C(7)H$_2$); 3.18 (t, 2 H, C(4)OC<u>H</u>$_2$(CH$_2$)$_2$CH$_3$, *J* = 6.1); 3.21 (s, 3 H, C(4)OMe); 5.13 (br.s, 1 H, C(1)OH); 5.56, 5.74 (both br.s, 1 H each, O=C(8)NH$_2$); 6.73 (s, 2 H, C(2)H, C(6)H) |
| **17** | 1.28, 1.29 (both t, 3 H each, both C(4)OCH$_2$C<u>H</u>$_3$, *J* = 7.0); 2.50 (s, 2 H, C(7)H$_2$); 3.24, 3.34 (both q, 2 H each, both C(4)OC<u>H</u>$_2$CH$_3$, *J* = 7.0); 5.10 (br.s, 1 H, C(1)OH); 5.62, 5.77 (both br.s, 1 H each, O=C(8)NH$_2$); 6.70 (s, 2 H, C(2)H, C(6)H) |
| **18** | 1.29 (t, 3 H, C(4)OCH$_2$C<u>H</u>$_3$, *J* = 7.0); 2.37 (br.s, 1 H, C(1)OH); 2.73 (s, 2 H, C(7)H$_2$); 3.22 (s, 3 H, C(4)OMe); 3.33 (q, 2 H, C(4)OC<u>H</u>$_2$CH$_3$, *J* = 7.0); 6.72 (s, 2 H, C(2)H, C(6)H) |
| **19** | 1.30 (t, 3 H, C(4)OCH$_2$C<u>H</u>$_3$, *J* = 7.0); 2.37 (br.s, 1 H, C(1)OH); 2.73 (s, 2 H, C(7)H$_2$); 3.21 (s, 3 H, C(4)OMe); 3.35 (q, 2 H, C(4)OC<u>H</u>$_2$CH$_3$, *J* = 7.0); 6.72 (s, 2 H, C(2)H, C(6)H) |
| **20** | 1.28 (t, 3 H, C(4)OCH$_2$C<u>H</u>$_3$, *J* = 7.0); 1.32 (t, 3 H, O=C(8)OCH$_2$C<u>H</u>$_3$, *J* = 7.2); 2.62 (s, 2 H, C(7)H$_2$); 3.21 (s, 3 H, C(4)OMe); 3.24 (q, 2 H, C(4)OC<u>H</u>$_2$CH$_3$, *J* = 7.0); 4.21 (br.s, 1 H, C(1)OH); 4.24 (q, 2 H, O=C(8)OC<u>H</u>$_2$CH$_3$, *J* = 7.2); 6.72 (s, 2 H, C(2)H, C(6)H) |
| **21** | 1.28 (t, 3 H, C(4)OCH$_2$C<u>H</u>$_3$, *J* = 7.0); 1.32 (t, 3 H, O=C(8)OCH$_2$C<u>H</u>$_3$, *J* = 7.2); 2.625 (s, 2 H, C(7)H$_2$); 3.14 (s, 3 H, C(4)OMe); 3.35 (q, 2 H, C(4)OC<u>H</u>$_2$CH$_3$, *J* = 7.0); 4.21 (br.s, 1 H, C(1)OH); 4.24 (q, 2 H, O=C(8)OC<u>H</u>$_2$CH$_3$, *J* = 7.2); 6.72 (s, 2 H, C(2)H, C(6)H) |
| **22** | 1.28, 1.285 (both t, 3 H each, both C(4)OCH$_2$C<u>H</u>$_3$, *J* = 6.9); 1.32 (t, 3 H, O=C(8)OCH$_2$C<u>H</u>$_3$, *J* = 7.1); 2.61 (s, 2 H, C(7)H$_2$); 3.23, 3.34 (both q, 2 H each, both C(4)OC<u>H</u>$_2$CH$_3$, *J* = 6.9); 4.17 (br.s, 1 H, C(1)OH); 4.24 (q, 2 H, O=C(8)OC<u>H</u>$_2$CH$_3$, *J* = 7.1); 6.68 (s, 2 H, C(2)H, C(6)H) |
| **23**** | 1.28 (t, 3 H, C(4)OCH$_2$C<u>H</u>$_3$, *J* = 6.9); 2.70 (s, 2 H, C(7)H$_2$); 3.21 (s, 3 H, C(4)OMe); 3.26 (q, 2 H, C(4)OC<u>H</u>$_2$CH$_3$, *J* = 6.9); 6.755 (s, 2 H, C(2)H, C(6)H) |
| **24**** | 1.28 (t, 3 H, C(4)OCH$_2$C<u>H</u>$_3$, *J* = 6.9); 2.70 (s, 2 H, C(7)H$_2$); 3.15 (s, 3 H, C(4)OMe); 3.355 (q, 2 H, C(4)OC<u>H</u>$_2$CH$_3$, *J* = 6.9); 6.755 (s, 2 H, C(2)H, C(6)H) |
| **25a** | 2.74, 2.82 (both d, 1 H each, C(7)H$_2$, *J* = 16.4); 2.88 (s, 1 H, C(1)OH); 3.46, 3.51 (both s, 3 H each, both C(4)OMe); 4.01 (d, 1 H, C(6)OH, *J* = 9.3); 4.32 (dd, 1 H, C(6)H, *J* = 4.6, *J* = 9.3); 4.47 (d, 1 H, C(5)H, *J* = 4.6); 6.50 (d, 1 H, C(2)H, *J* = 1.2) |
| **26** | 2.465 (br.s, 1 H, C(3a)OH); 2.72, 2.91 (both d, 1 H each, C(3)H$_2$, *J* = 18.6); 3.33, 3.43 (both s, 3 H each, both C(4)OMe); 4.41 (d, 1 H, C(7)H, *J* = 3.2); 5.02 (d, 1 H, C(7a)H, *J* = 3.2); 6.57 (s, 2 H, C(4)H) |
| **27** | 1.18 (t, 3 H, C(6)OCH$_2$C<u>H</u>$_3$, *J* = 7.0); 2.45 (br.s, 1 H, C(3a)OH); 2.71, 2.92 (both d, 1 H each, C(3)H$_2$, *J* = 18.5); 3.43 (s, 3 H, C(6)OMe); 3.49, 3.62 (both m, 1 H each, C(6)OC<u>H</u>$_2$CH$_3$); 4.43 (d, 1 H, C(7)H, *J* = 3.3); 5.03 (d, 1 H, C(7a)H, *J* = 3.3); 6.55 (s, 2 H, C(4)H) |
| **28** | 2.65, 2.99 (both d, 1 H each, C(3)H$_2$, *J* = 18.2); 3.37, 3.43 (both s, 3 H each, both C(4)OMe); 4.74 (d, 1 H, C(7)H, *J* = 4.6); 4.95 (d, 1 H, C(7a)H, *J* = 4.6); 6.46 (s, 2 H, C(4)H) |
| **29**** | 2.28, 2.35 (both d, 1 H each, C(7)H$_2$, *J* = 15.1); 3.60 (s, 3 H, C(4)OMe); 3.95 (s, 1 H, C(6)H); 6.28 (s, 1 H, C(2)H) |

* $^1$H NMR spectra were recorded at 700 MHz for compounds **13**, **14**, **26**, **27** (in $CDCl_3$), at 500 MHz for compounds **28** (in $CDCl_3$) and **29** (in DMSO-$d_6$), and at 300 MHz for compounds **15—19** and **25a** (in $CDCl_3$); the signals were assigned using the HMBC two-dimensional experiment.

** No signals for the hydroxyl protons were observed.

The peaks of pseudomolecular ions in the (+)-ESI mass spectra of derivatives **20** and **21** having a $CH_2COOEt$ side moiety correspond to the molecular formula $C_{13}H_{18}Br_2O_5$. The EI mass spectra of these compounds contain a strong peak for the fragment ion at *m/z* 327 $[M - CH_2COOCH_2CH_3]^+$, whereas the HMBC spectra exhibit correlations between the C(8) atom and protons of methylene ($H_2C(7)$) and ester (—OC<u>H</u>$_2$CH$_3$) units. The

**Table 2.** $^{13}C$ NMR spectra of compounds **13** and **16—19** ($CDCl_3$)*

| Atom or group | δ | | | | |
|---|---|---|---|---|---|
| | **13** | **16** | **17** | **18** | **19** |
| C(1) | 70.9 | 70.8 | 70.9 | 70.0 | 70.0 |
| C(2)H, C(6)H | 139.5 | 138.9 | 138.9 | 137.6 | 137.7 |
| C(3), C(5) | 124.4 | 124.2 | 125.2 | 127.4 | 127.3 |
| C(4) | 96.2 | 96.0 | 95.6 | 95.7 | 95.7 |
| C(7)$H_2$ | 43.8 | 43.5 | 43.8 | 30.1 | 30.1 |
| C(8) | 172.4 | 171.8 | 172.3 | 114.9 | 115.0 |
| C(4)O<u>Me</u> | 51.2 | 51.2 | — | 51.2 | 51.4 |
| C(4)O<u>Et</u> | 15.1 | — | 59.1, 15.0<br>59.5, 15.1 | 60.0, 15.0 | 59.9, 15.0 |
| C(4)O<u>Bu</u> | — | 63.5, 31.2,<br>19.4, 13.7 | — | — | |

* $^{13}C$ NMR spectra were recorded at 75 MHz for compounds **13** and **18**, at 125 MHz for compounds **17** and **19**; $^{13}C$ NMR spectrum of compound **16** was obtained through the CH-correlation in the HMBC spectrum; the signals in the spectra of compounds **13**, **16**, **18**, and **19** were assigned using the HMBC two-dimensional experiment.

NOESY spectrum of ethyl (*E*-3,5-dibromo-4-ethoxy-1-hydroxy-4-methoxycyclohexa-2,5-dien-1-yl)acetate (**20**) has a cross-peak between the $H_2C(7)$ ($\delta_H$ 2.62, s) and —OC<u>$H_2$</u>$CH_3$ at C(4) ($\delta_H$ 3.24, q). Conversely, the cross-peaks between the $H_2C(7)$ ($\delta_H$ 2.63, s) and —$OCH_3$ ($\delta_H$ 3.14, s), as well as between the —OH ($\delta_H$ 4.22, br.s) and —OC<u>$H_2$</u>$CH_3$ ($\delta_H$ 3.35, q) are found in the NOESY spectrum its *Z*-isomer (**21**).

In the HMBC spectrum of homologous diethyl ketal **22**, the protons of both ethoxy groups —OC<u>$H_2$</u>$CH_3$ correlate to C(4). The data of its $^1H$ and $^{13}C$ NMR spectra (Tables 1 and 3) and (+)-ESI mass spectrum (molecular formula $C_{14}H_{20}Br_2O_5$) correspond to the structure of ethyl (3,5-dibromo-4,4-diethoxy-1-hydroxycyclohexa-2,5-dien-1-yl)-acetate.

**Table 3.** $^{13}C$ NMR spectra of compounds **20—22** and **24** ($CDCl_3$)*

| Atom or group | δ | | | |
|---|---|---|---|---|
| | **20** | **21** | **22** | **24** |
| C(1) | 70.2 | 70.2 | 70.3 | 70.0 |
| C(2)H, C(6)H | 139.3 | 139.3 | 138.9 | 138.5 |
| C(3), C(5) | 124.4 | 124.7 | 125.3 | 124.8 |
| C(4) | 96.2 | 96.1 | 95.6 | 95.8 |
| C(7)$H_2$ | 43.9 | 43.0 | 43.6 | 42.8 |
| C(8) | 170.6 | 170.9 | 171.3 | 172.0 |
| C(4)O<u>C</u>$H_3$ | 50.9 | 50.8 | — | 50.8 |
| C(4)<u>C</u>$H_2CH_3$ | 59.2 | 59.4 | 59.3, 59.3** | 59.5 |
| C(4)$CH_2$<u>C</u>$H_3$ | 15.1 | 14.4 | 14.7, 14.7** | 14.9 |
| C(8)OO<u>C</u>$H_2CH_3$ | 61.7 | 61.6 | 61.7 | — |
| C(8)OO$CH_2$<u>C</u>$H_3$ | 13.5 | 14.1 | 14.0 | — |

* $^{13}C$ NMR spectra were obtained through the CH-correlations in the HMBC spectra.

** In the HMBC spectrum of compound **22**, the corresponding signals for the carbon atoms of two ethoxyls at C(4) were undistinguished.

The $^1H$ and $^{13}C$ NMR spectra of isomeric acids **23** and **24** (see Tables 1 and 3) are close to the corresponding spectra of their derivatives **20** and **21**, however, they have no signals for the ester ethoxyl. These data together with the results of the (+)- and (−)-ESI mass spectrometric studies allows us to draw a conclusion that molecular formula of compounds **23** and **24** is $C_{11}H_{14}Br_2O_5$. The IR spectra of compounds **23** and **24** exhibit a broad absorption band at 3300—2400 $cm^{-1}$ characteristic of the stretching vibrations of hydroxy group of carboxylic acid dimers. The NOESY spectrum of (*E*-3,5-dibromo-4-ethoxy-1-hydroxy-4-methoxycyclohexa-2,5-dien-1-yl)acetic acid (**23**) contains a cross-peak between the $H_2C(7)$ ($\delta_H$ 2.70, s) and —OC<u>$H_2$</u>$CH_3$ ($\delta_H$ 3.26, q), whereas the corresponding spectrum of its *Z*-isomer (**24**) — between the $H_2C(7)$ ($\delta_H$ 2.70, s) and —$OCH_3$ ($\delta_H$ 3.15, s).

Note that earlier attempts to separate mixtures of stereoisomeric dibromotyrosine mixed ketals were unsuccessful. Because of this, aplysinketal A (**15**) isolated by recrystallization still is the only dibromotyrosine ketal with the known stereochemistry found by X-ray diffraction analysis.[10]

The isolated pairs of stereoisomeric mixed ketals are examples of a rare type of geometrical isomers. Their molecules are achiral, since they have a plane of symmetry, in which substituents at the two saturated carbon atoms of the cycle are placed. This plane of symmetry goes through the atoms C(1) and C(4) perpendicular to the plane of the double bonds of the ring. The isomers obtained can be distinguished based on the specificities of their $^1H$ NMR spectra. Thus, the signals for the protons of the ester groups at C(4) atom in the $^1H$ NMR spectrum of *E*-isomer are closer as compared to the corresponding signals in the

**Table 4.** The chemical shift values of the signals for the α-protons of the alkoxy groups at the C(4) atom in the $^1$H NMR spectra ($CDCl_3$) of configurational isomers **13—16**, **18—21**, **23**, and **24***

| Compound | δ (*J*/Hz) | |
|---|---|---|
| | $H_3COC(4)$ (c) | $-H_2COC(4)$ |
| *E*-**13** | 3.21 | 3.27 (q, *J* = 6.7) |
| *Z*-**14** | 3.16 | 3.36 (q, *J* = 6.7) |
| *Z*-**15** | 3.16 | 3.28 (t, *J* = 6.1) |
| *E*-**16** | 3.21 | 3.18 (t, *J* = 6.1) |
| *E*-**18** | 3.22 | 3.33 (q, *J* = 7.0) |
| *Z*-**19** | 3.21 | 3.35 (q, *J* = 7.0) |
| *E*-**20** | 3.21 | 3.24 (q, *J* = 7.0) |
| *Z*-**21** | 3.14 | 3.35 (q, *J* = 7.0) |
| *E*-**23** | 3.21 | 3.26 (q, *J* = 6.9) |
| *Z*-**24** | 3.15 | 3.355 (q, *J* = 6.9) |

* $^1$H NMR spectra were recorded at 300 MHz for compounds **15**, **16**, **18**, **19** and at 700 MHz for the rest of compounds.

spectrum of *Z*-isomer (Table 4). This difference in CS, as well as the difference in the signals for the protons of similar substituents at C(4) atom in the $^1$H NMR spectra of dimethoxy ketals **7** and **12** and diethoxy ketals **17** and **22**, are explained by the ability of different substituents at C(1) atom to differently shield the *cis*-alkoxy groups at C(4) atom.

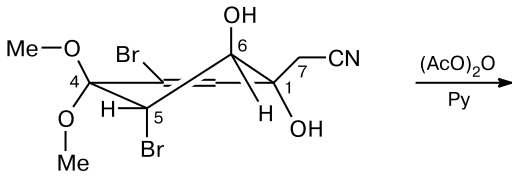
**Ketals of *p*-hydroxycyclohexenone derivatives.** Ketals **25—28** belong to the earlier unknown type of monoene ketals from extracts of verongid sponges.

The (+)-ESI mass spectrum of compound **25a** contains a peak at *m/z* 391.9074 $[M + Na]^+$ corresponding to the molecular formula $C_{10}H_{13}Br_2NO_4$. The $^1$H—$^1$H COSY spectrum of compound **25a** shows correlation between the vicinal protons H(6) and H(5), as well as a cross-peak between the olefin proton H(2) and the equatorial proton H(6) with characteristic W-arrangement; the HMBC spectrum exhibits correlational peaks between the ketal C(4) and the protons of two $-OCH_3$ groups, H(2) and H(5). Despite that the signal for the —C≡N group is not observed in the $^{13}$C NMR spectra because of the small amount of isolated compound, the presence of the nitrile is indicated by the value of CS and splitting of the signals for the neighboring $H_2C(7)$ group in the $^1$H (see Table 1) and $^{13}$C NMR spectra, which are characteristic of the corresponding spectra of aeroplysinin-1 (**1**).

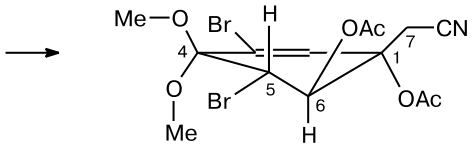
Though analysis of the NOESY spectrum of compound **25a** and the absence of the data on the closely related structures do not allow us to unambiguously establish configuration of the C(5) atom, results of acetylation of this ketal are very informative. Thus, the $^1$H NMR spectrum of compound **25a** exhibits the spin-spin coupling constant for two vicinal (presumably, diequatorial or axial-equatorial) protons H(5) and H(6) of the ring equal to 4.6 Hz, whereas in the $^1$H NMR spectrum of diacetate **25c** it increases to 11.3 Hz. Obviously, acetylation of compound **25a** causes changes of the half-chair conformation of the cyclohexene ring (see Ref. 14 and references cited therein), which results in the fact that diequatorial H(5) and H(6) atoms become diaxial due to the introduction of bulky *O*-acetyl groups at the neighboring positions. In this case, acetylation of the hydroxyl at HC(6) is a decisive factor for the change of the ring conformation, which is indicated by the $^1$H NMR spectrum of monoacetate **25b** with $J_{5,6}$ = 10.5 Hz.

According to the results of our quantum chemical calculations (performed based on the literature data[15,16]), the cyclohexene rings of compounds **25a** and **25c** have conformations close to the half-chair $^6HC_5$ and half-boat $HB_6$, respectively. Therefore, it appears that no complete inversion of the ring $^6HC_5 \rightarrow {}^5HC_6$ take place upon acetylation (Scheme 1). The calculated values of the dihedral angle between the vicinal H(5) and H(6) atoms for compounds **25a** (64.3°) and **25c** (175.3°) in the corresponding conformations agree with the values of spin-spin coupling constants of these protons observed in the $^1$H NMR spectra of the ketal and its diacetate.

**Scheme 1**




**25a**




**25c**

Domination of different conformations of the cyclohexene ring in solutions of diol ketal **25a** and its diacetate **25c** was confirmed by the change in intensity of responses of $H_2C(7)$ protons to the H(6) and H(5) in the NOESY experiments. Thus, in the NOESY spectrum of diol **25a** intensity of the correlation peak between the $H_2C(7)$ and H(6) is higher than between the $H_2C(7)$ and H(5), whereas in the corresponding spectrum of diacetate **25c** the picture is the opposite. To sum up, the diequatorial positions of the H(5) and H(6) atoms in diol **25a** indicates the *S*-configuration of C(5). Therefore, the structure of [(1*S*,5*S*,6*R*)-

3,5-dibromo-1,6-dihydroxy-4,4-dimethoxycyclohex-2-en-1-yl]acetonitrile (**25a**) corresponds to the compound obtained.

Signals for the substituted hydroxylated ring of γ-lactone in the $^1$H (see Table 1) and $^{13}$C NMR spectra and IR spectra of dimethyl ketal **26** and aeroplysinin-2 (**2**)[11] looks alike. The HMBC spectrum of compound **26** exhibits correlation peaks between the two —$OCH_3$ groups and the ketal C(6), and in this case the olefin (H(4)) and the vicinal (H(7) and HC(7a)) protons also respond to the C(6). The NOESY spectrum of the compound under consideration contains a cross-peak between the *cis*-oriented H(3β) at $\delta_H$ 2.72 (d, $J$ = 18.6 Hz) and H(7) at $\delta_H$ 4.41 (d, $J$ = 3.2 Hz) protons, that together with the EI mass spectrometric data correspond to the structure of (3a*S*,7*S*,7a*R*)-5,7-dibromo-3a-hydroxy-6,6-dimethoxy-3a,6,7,7a-tetrahydro-1-benzofuran-2(3*H*)-one (**26**).

The NMR spectra, IR and EI mass spectra of compound **27** indicate that it is a homolog of ketal **26**. The key NOESY correlations for (3a*S*,6*R*,7*S*,7a*R*)-5,7-dibromo-6-ethoxy-3a-hydroxy-6-methoxy-3a,6,7,7a-tetrahydro-1-benzofuran-2(3*H*)-one (**27**) include the cross-peaks between the H(3β) at $\delta_H$ 2.71 (d, $J$ = 18.5 Hz) and H(7) at $\delta_H$ 4.43 (d, $J$ = 3.3 Hz), —$OC\underline{H}_2CH_3$— at $\delta_H$ 3.49 (m), —$OCH_2C\underline{H}_3$ at $\delta_H$ 1.18 (t, $J$ = 7.0 Hz).

Ketals **26** and **28** have the same molecular weight and fragmentation in the EI mass spectra. The main difference in the NMR spectra of compounds **26** and **28** as epimers at the C(7) atom consists in the position of signals for the HC(7) proton and carbon. Thus, in the $^{13}$C NMR spectrum of compound **28** the signal for C(7) is shifted by 5.2 ppm to the low field as compared to the corresponding signal for C(7) in the spectrum of epimer **26**. In fact, according to our quantum chemical calculation in the $^{13}$C NMR spectrum of compound **28** (conformation of the cyclohexene ring is close to the half-boat $^7$HB) the signal for the C(7) atom should be downfield shifted by 9.4 ppm as compared to the corresponding signal in analogous spectrum of epimer **26** (the half-chair conformation $^{7a}HC_7$). In addition, in the $^1$H NMR spectrum of compound **26** (see Table 1) the difference in CS of the signals for the $H_2C(3)$ geminal protons in the lactone ring is 0.19 ppm, whereas for compound **28** it is 0.34 ppm. Such an increase in the nonequivalence of methylene protons in epimer **28** can be explained by the effect of their approximation to the pseudoaxial bulky bromine. In the IR spectra, the absorption band of the lactone carbonyl group (1797 $cm^{-1}$) of compound **28** is shifted toward somewhat higher frequencies than for compounds **26** and **27** (1792 and 1791 $cm^{-1}$, respectively). This suggests that in (3a*S*,7*R*,7a*R*)-5,7-dibromo-3a-hydroxy-6,6-dimethoxy-3a,6,7,7a-tetrahydro-1-benzofuran-2(3*H*)-one (**28**), the electronegative bromine is closer to the carbonyl group (the known "field effect" or dipolar interaction).

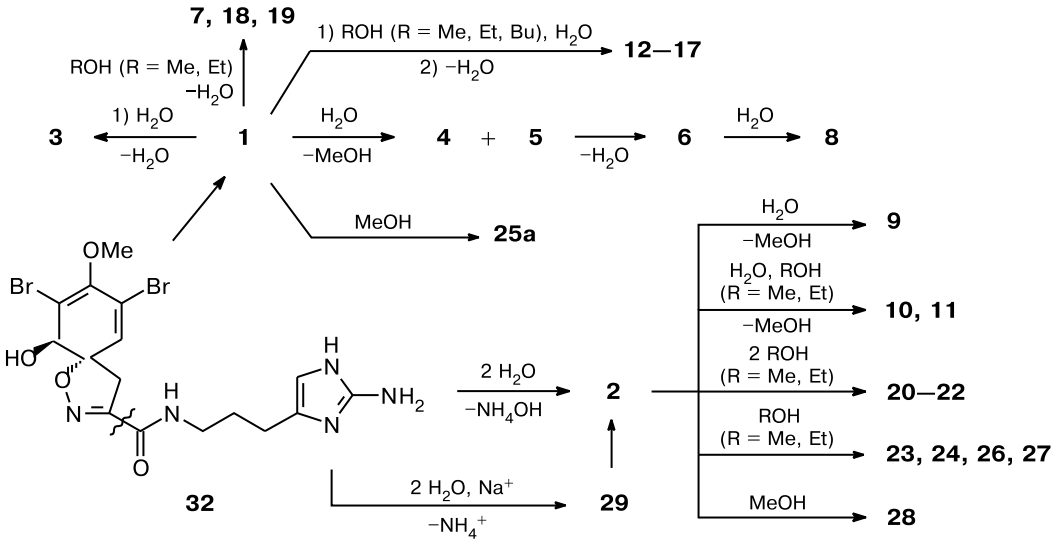
Ketals **26—28** are unstable and easily convert to keto acid **9**, which is characteristic of aeroplysinin-2 (**2**) as well.[11]

**Cyclohexadienol derivative.** The $^{13}$C NMR spectra of compound **29** and its known analog with the acetamide side chain[4] are close. However, the presence of the absorption band at 1581 $cm^{-1}$ in the IR spectrum of compound **29** indicates that it contains a carboxylate group. In addition, the peak of pseudomolecular ion in the (−)-ESI mass spectrum of this compound at $m/z$ 354.8834 $[M]^-$ corresponds to the molecular formula $C_9H_9O_5Br_2$. The presence of a carboxylate at position 8, secondary hydroxyl at CH(6), and tertiary hydroxyl at C(1) was demonstrated by the HMBC correlation and confirmed by the results of acetylation of compound **29**. Thus, treatment of this compound with acetic anhydride in pyridine at −17 °C gives a mixture of aeroplysinin-2 (**2**) and its acetate[11] (2 : 1) identified using $^1$H NMR and HMBC spectra, IR and EI mass spectra. Moreover, the HMBC spectrum of [(1*S*,6*R*)-3,5-dibromo-1,6-dihydroxy-4-methoxycyclohexa-2,4-dien-1-yl]acetate (**29**) shows the presence of admixture of aeroplysinin-2 (**2**) in it as a result of ready transformation of compound **29** to γ-lactone **2**.

**On origin of isolated compounds.** Isoxazoline alkaloids including aerophobin-2 (**32**) capable to be cleaved to compounds **1** and **8** when sponges tissues are injured[2] (Scheme 2) have been found by us earlier[17] in the extract of *Aplysina* sp. If the product of "activated chemical defense" aeroplysinin-1 (**1**) is a precursor of other simple dibromotyrosines found in the extract, then numerous enones and ketals mentioned above should be formed after addition of water or ethanol, respectively, to the double bond of the fragment of enol ether **1**.

In fact, hydrolysis of compound **1** occurs under mild conditions. Thus, when the $^1$H NMR spectrum of aeroplysinin-1 (**1**) is recorded in $CDCl_3$ at room temperature, the disappearance of usual residual signal for water protons and appearance of an intense signal for the protons of free methanol and signals for the protons of compounds **4** and **5** are observed with time. The $^1$H NMR spectrum of the hydrolysis products of **1**, accelerated by heating to 50—55 °C in $CDCl_3$, contains signals not only for compounds **4** and **5**, but also for *p*-hydroxycyclohexadienones **6** and **8**. It is obvious that dehydration of compounds **4** and **5** to dienone **6**, then giving **8**, is facilitated by the presence of the labile H(5) atom at α-position to the hydroxy group at the C(6) atom. Rechromatographic conditions for aeroplysinin-1 (**1**) upon the reversed-phase HPLC in aqueous methanol also lead to the formation of compounds **4—6** and **8**. To sum up, addition of water to the enol ether fragment of aeroplysinin-1 (**1**) does not require participation of *O*-dealkylating enzymes, as it has been suggested earlier.[18] In addition, it is obvious that hydration of the nitrile group of compound **1** occurs rather readily and does not require interfering postulated by nitrile hydratases.[18]

**Scheme 2**




The fact that rechromatography of aeroplysinin-1 (**1**) in aqueous methanol in addition gives ketals **7** and **12**, whereas in aqueous ethanol ketals **13** and **14**, indicates that addition of alcohols to compound **1** can also proceed under rather mild conditions. However, storage of aeroplysinin-1 (**1**) in $CD_3OD$ over two months shows no signs of its significant transformation to the corresponding ketal. Only acidification of a solution of aeroplysinin-1 (**1**) in $CD_3OD$ with HCl after heating at 55 °C gives rise to ketals with the nitrile, amide, and carboxy groups in the side chain and two $—OCD_3$ residues at the C(4) atom as follows from the analysis of the $^1H$, HMBC NMR spectra and the EI mass spectra. It is obviously that transketalization takes place in acidic medium[19] with the exchange of the methoxyl with $—OCD_3$. Thus, aeroplysinin-1 (**1**) can be a precursor of ketals, rather than hypothetical compounds **30** and **31**. The presence of a number of new compounds also indicates formation of ketals from enol ethers *Aplysina* sp. For instance, ketal **25a** can be a product of addition of methanol to compound **1**, whereas ketals **26—28** can be products of addition of the corresponding alcohol to aeroplysinin-2 (**2**).

Diethyl ketals **17** and **22** are apparently products of ethanolysis of either the starting enol ether, or the corresponding methyl ethyl ketals. At least, we found the corresponding signs of transformation of aeroplysinin-2 (**2**) upon rechromatography of its ethanol solution in aqueous ethanol, which showed that one of the fractions obtained contained, together with diethyl ketal **22**, a homolog of aeroplysinin-2 (**2**) with the ethoxyl instead of methoxyl at the corresponding position. The presence of ethoxy analog of compound **2** was indicated by the $^1H$, HMBC NMR and IR data, but, unfortunately, we were unable to register the mass spectrum of this derivative due to its lability. Apparently, ethoxy analog of aeroplysinin-2 (**2**) in the presence of ethanol is transformed with the lactone ring opening to diethyl ketal **22**, whereas aeroplysinin-2 (**2**) itself — to methyl ethyl ketals **20** and **21** (while in the presence of water — to keto acid **9**).[11]

Compound **29** can be considered as the final hydrolysis product of aeroplysinin-1 (**1**) at the nitrile group, however, it is doubtful that such a deep hydrolysis of compound **1** can occur under mild enough conditions of isolation, while the labile enol fragment remained intact. The more real seems formation of acid derivative **29** in the step of isoxazoline ring opening in the precursor of aerophobin-2 (**32**) type after addition of two water molecules and exchange of counter ion $NH_4^+$ with sodium. Since aeroplysinin-2 (**2**) can simultaneously be formed in this process through lactonization, then no postulation of hypothetical imino ether **31** is required to explain the origin of **2**.

Scheme 2 illustrates a suggested sequence of transformations leading from isolated alkaloids to their derivatives.

In conclusion, isoxazoline alkaloids and product of their bioconversion, aeroplysinin-1 (**1**),[2] can play a key role in the formation of simple dibromotyrosine derivatives (enones, dienones, ketals, and lactones) isolated from the extracts of sponges of the family *Aplysina*. In this case, existence of the mechanism of activated chemical defense in *Aplysina* assumes that all the simple dibromotyrosines of its extracts are not constitutive. However, can they be considered as artifacts? Conditions used for the isolation allowed us to obtain a set of compounds typical of the extracts of *Aplysina*. All the ketals, esters **10** and **11**, which showed no antimicrobal activity,[10,20,21] are obvious artifacts, since they contain residues of alcohols used in the isolation process. This question cannot be answered unambiguously for other simple dibromotyrosines, since they

can be formed on injuring the sponge tissues under natural conditions and, as a rule, possess antibacterial properties.[6,7,13,21,22] The formation of numerous related antibacterial compounds from the labile precursors after addition/elimination of water can be "programmed" by requirements of the sponge organisms: a wide spectrum of structures of these antibiotics decreases the risk of development of resistance to them in microbs.

## Experimental

$^1$H and $^{13}$C NMR spectra were recorded on Bruker DPX-300, Bruker DRX-500, and Bruker Avance III 700 spectrometers (300.13, 500.13, and 700.13 MHz for the $^1$H NMR spectra, 75.48 and 125.75 MHz for the $^{13}$C NMR spectra), $Me_4Si$ was used as an internal standard. Optical rotation was measured on a Perkin—Elmer 343 polarimeter; specific rotation is given in deg mL g$^{-1}$ dm$^{-1}$, the concentration of solution is given in g (100 mL)$^{-1}$. IR spectra were recorded on Bruker Vector 22 and Bruker Equinox 55 Fourier-spectrophotometers with the resolution 2 cm$^{-1}$ in $CDCl_3$ in $CaF_2$ cuvettes (the layer width was 0.61—1.00 mm) or in KBr pellets. Measurements of frequencies and values of expansion of the contour of the stretching vibrations absorption bands ν(OH) to the components were performed using the OPUS/IR 02 program package the 3.0.2 version; reproduction of frequency measurements was at least 0.5 cm$^{-1}$. UV spectra were recorded on a Shimadzu UV-1601PC spectrophotometer in methanol, EI mass spectra were recorded on a AMD-604S instrument. Mass spectra ESI were recorded on a quadruple-time-of-flight mass spectrometer with the double source of electrospray ionization 6510 (Agilent, USA), the needle potential was 4 kV in the negative and 3.5 kV in the positive modes of recording of ions at the temperature of the drying gas 325 °C and potential of fragmentator 243 V. Quantum chemical calculations were accomplished using the functional density theory with the B3LYP exchange correlation functional (see Ref. 15) in the 6-31G(d) and 6-311G(d) basis of atomic orbitals using the GAUSSIAN 03 program.[16] Electron energies ($E$) and constants of magnetic shielding of hydrogen and carbon atoms were found by the complete optimization of the geometry in the 6-311G(d) basis of atomic orbitals in the ground electron state of compounds. Corrections for the energy of zero vibrations (ZPE), temperature corrections $G_T$ and $H_T$ were calculated in the same 6-311G(d) basis. The Gibbs energy $G$ and enthalpy $H$ were calculated with allowance for all the electronic, translational, rotational, and vibrational degrees of freedom at 298.15 K. Column chromatography was carried out on Sephadex LH-20 (25—100 μm, Pharmacia, Sweden) and silica gel L (40/100 μm, Chemapol, Czechoslovakia), TLC was performed on Sorbfil plates (Sorbpolimer, Krasnodar, Russia) with the foil-bound STKh-1A (5—17 μm) silica gel layer. HPLC was performed on a Du Pont Series 8800 Instrument chromatograph with the RIDK-102 refractometer as a detector on the ZORBAX Carbohydrate (4.6×250 mm) columns for compound **29**, Supelco Discovery®C8 (4.6×250 mm) for compounds **4**, **5**, **8**, and **12**, and Agilent ZORBAX Eclipse XDB-C8 (4×150 mm) for the rest of compounds in the mixtures of MeOH, EtOH, or MeCN with water.

**Biological material:** The sponge *Aplysina* sp. (order Verongida, family Aplysinidae) was collected by scuba at the depth 20—38 m near Ladd Reef in the South China Sea (8°39,9 N, 111°41,6 E) during a cruise of the R/V "Academik Oparin" in May, 2007. The sponge was identified by V. B. Krasokhin (PIBOC FEB RAS, Vladivostok, Russian Federation). A voucher specimen (034—164) is deposited with the Pacific Institute of Bioorganic Chemistry collection, Vladivostok, Russia. The ethanol extract of *Aplysina* sp. exhibited antibacterial and antifungal activity.

**Isolation of compounds 1—29.** The collected sponge material (~4 kg of crude weight) was frozen, stored at −15 °C and then cut and extracted with ethanol at room temperature. The extract was concentrated *in vacuo*, the residue was dissolved in water and extracted with *n*-hexane and then with *n*-butanol. The sequence of isolation of compounds **1**—**29** from the butanol fraction of the sponge is given in Scheme 3.

**Acetylation of compound 25a.** Pyridine (0.2 mL) and acetic anhydride (0.2 mL) were added to compound **25a** (0.2 mg), the solution was cooled to −17 °C and kept for 3 days. A mixture of monoacetate **25b** and diacetate **25c** in the ratio ~7 : 10 was obtained after concentration at 55 °C. For exhaustive acetylation, the same amounts of Py and $Ac_2O$ were added to this mixture, which was kept for ~14 h at +3 °C.

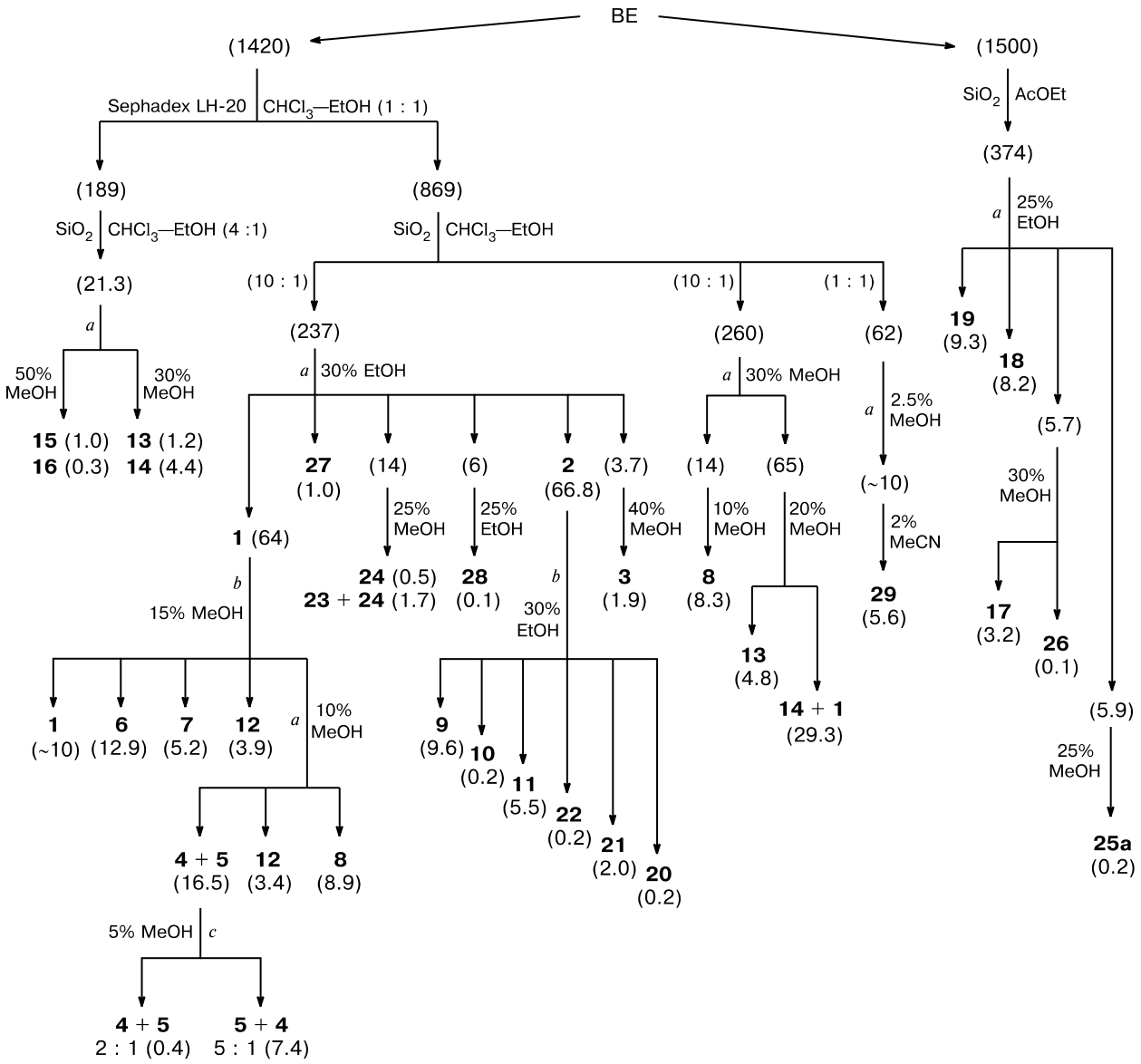
**Acetylation of compound 29.** Pyridine (0.2 mL) and acetic anhydride (0.2 mL) were added to compound **29** (0.5 mg), the solution was cooled to −17 °C and kept for 44 h, then concentrated to dryness *in vacuo* at 55 °C to obtain a mixture of aeroplysinin-2 (**2**) and aeroplysinin-2 acetate (~2 : 1).[21]

**Alcoholysis of aeroplysinin-1 (1).** Methanol-$d_4$ (0.6 mL) and concentrated HCl (2 drops) were added to compound **1** (0.5 mg), the solution in the NMR tube was heated for 5.5 h at 55 °C to obtain a mixture of ketones, ketals, and the starting compound in the ratio 1 : 1.5 : 2.

**2-(*E*-3,5-Dibromo-4-butoxy-1-hydroxy-4-methoxycyclohexa-2,5-dien-1-yl)acetamide (16)**, amorphous compound. IR ($CDCl_3$), ν/cm$^{-1}$: 3523, 3406 ($NH_2$), 3362 (OH), 1682 (C=O), 1099, 1079 (OMe, OBu). $^1$H and $^{13}$C NMR spectra ($CDCl_3$) of compounds **16**—**19** are given in Tables 1 and 2, respectively. HMBC ($CDCl_3$, 500 MHz), H/C ($\delta_H$, $\delta_C$ HC(2) and HC(6), C(3) and C(4) overlap): 2 (6)/3 (5), 4, 6 (2), 7; 7/1, 2, 6, 8; $OCH_3$/4; $OC\underline{H}_2(CH_2)_2CH_3$/4, $OCH_2C\underline{H}_2C\underline{H}_2CH_3$; $OCH_2C\underline{H}_2CH_2CH_3$/$OC\underline{H}_2CH_2C\underline{H}_2C\underline{H}_3$; $O(CH_2)_2C\underline{H}_2CH_3$/$OC\underline{H}_2(CH_2)_2C\underline{H}_3$; $O(CH_2)_3C\underline{H}_3$/$OCH_2C\underline{H}_2C\underline{H}_2CH_3$. COSY ($CDCl_3$, 700 MHz), H/H: $O(CH_2)_3C\underline{H}_3$/$O(CH_2)_2C\underline{H}_2CH_3$; $O(CH_2)_2C\underline{H}_2CH_3$/$OCH_2C\underline{H}_2CH_2C\underline{H}_3$; $OCH_2C\underline{H}_2CH_2CH_3$/$OC\underline{H}_2CH_2C\underline{H}_2CH_3$; $OC\underline{H}_2(CH_2)_2CH_3$/$OCH_2C\underline{H}_2CH_2CH_3$. MS (EI, 70 eV), $m/z$ ($I_{rel}$ (%)): 380/382/384 [M − MeO]$^+$ (4/10/4), 338/340/342 [M − BuO]$^+$ (15/28/12), 306/308/310 [M − MeO − − BuO − H]$^+$ (13/25/15), 293/295/297 (8/18/13), 274/276/278 (22/33/22), 242/244 (33/47), 215/217 (33/48), 185/187 (19/18), 59 (100), 32 (70). MS (+)-ESI, $m/z$: 433.9562 [M + Na]$^+$. $C_{13}H_{19}{}^{79}Br_2NO_4Na$. Calculated: $m/z$ 433.9579.

**2-(3,5-Dibromo-4,4-diethoxy-1-hydroxycyclohexa-2,5-dien-1-yl)acetamide (17)**, amorphous compound. IR ($CDCl_3$), ν/cm$^{-1}$: 3523, 3406 ($NH_2$), 3362 (OH), 1680, 1591 ($NH_2$), 1113, 1089, 1062, 1033 (OEt). MS (EI, 70 eV), $m/z$ ($I_{rel}$ (%)): $m/z$ 397/399/401 [M]$^+$ (0.5/1/0.5), 352/354/356 [M − EtO]$^+$ or [M − $CONH_2$ − − H]$^+$ (45/88/45), 334/336/338 [M − EtO − $H_2O$]$^+$ (10/19/11), 318/320 [M − Br]$^+$ (26/25), 307/309/311 [M − 2 EtO]$^+$ (22/40/26), 293/295/297 (10/17/9), 279/281/283 (14/27/17), 272/274 [M − EtO − HBr]$^+$ (50/61), 256/258 (72/71), 244/246 (44/44), 229/231 (100/99), 201/203 (58/63), 185/187 (35/34), 59 (49).

**Scheme 3***




*a*. HPLC; *b*. HPLC of the fraction after storage; *c*. HPLC, then recrystallization ($CHCl_3$—hexane).

* The weight (mg) is given in parentheses, BE is a butanol extract.

MS (+)-ESI, *m/z*: 419.9429 $[M + Na]^+$. $C_{13}H_{19}{}^{79}Br_2NO_4Na$. Calculated: *m/z* 419.9422.

**(*E*-3,5-Dibromo-4-ethoxy-1-hydroxy-4-methoxycyclohexa-2,5-dien-1-yl)acetonitrile (18)**, amorphous compound. IR ($CDCl_3$), $\nu/cm^{-1}$: 3586 (OH), 2836 (OMe), 1659, 1629 (*cis*-C=C), 1110, 1093, 1063, 1026, 1017 (OMe, OEt). (The absorption band of the nitrile group overlaps with the absorption of $CDCl_3$, that is observed in the IR spectra of all the dibromotyrosine nitriles studied.) HMBC ($CDCl_3$, 300 MHz), H/C: 2 (6)/3 (5), 4, 6 (2), 7; 7/1, 2, 6, 8; $OCH_3$/4; $OC\underline{H}_2CH_3$/4, $OCH_2\underline{C}H_3$; $OCH_2C\underline{H}_3$/$O\underline{C}H_2CH_3$. MS (EI, 70 eV), *m/z* ($I_{rel}$ (%)): *m/z* 365/367/369 $[M]^+$ (2/4/2), 334/336/338 $[M - MeO]^+$ (29/63/38), 320/322/324 $[M - EtO]^+$ (28/53/26), 304/306/308 (15/29/17), 294/296/298 $[M - CH_2CN - MeO]^+$ (21/37/19), 286/288 $[M - Br]^+$ (73/73), 280/282/284 (59/97/50) $[M - CH_2CN - EtO]^+$, 265/267/269 (41/82/49), 255/257 $[M - MeO - Br]^+$ (97/97), 246/248 $[M - CH_2CN - Br]^+$ (92/94), 241/243 $[M - EtO - Br]^+$ (100/100), 227/229 (69/64), 185/187 (27/30). MS (+)-ESI, *m/z*: 387.9168 $[M + Na]^+$. $C_{11}H_{13}{}^{79}Br_2NO_3Na$. Calculated: *m/z* 387.9160.

**(*Z*-3,5-Dibromo-4-ethoxy-1-hydroxy-4-methoxycyclohexa-2,5-dien-1-yl)acetonitrile (19)**, amorphous compound. IR ($CDCl_3$), $\nu/cm^{-1}$: 3586 (OH), 2834 (OMe), 1658, 1629 (*cis*-C=C), 1108, 1090, 1065, 1027, 1018 (OMe, OEt). MS (EI, 70 eV), *m/z* ($I_{rel}$ (%)): *m/z* 365/367/369 $[M]^+$ (1/2/1), 334/336/338 $[M - MeO]^+$ (5/13/9), 320/322/324 $[M - EtO]^+$ (17/34/17), 304/306/308 (6/10/5), 294/296/298 $[M - CH_2CN - MeO]^+$ (7/12/7), 286/288

$[M - Br]^+$ (35/35), 280/282/284 (31/51/25) $[M - CH_2CN - EtO]^+$, 265/267/269 (23/46/27), 255/257 $[M - MeO - Br]^+$ (38/38), 246/248 $[M - CH_2CN - Br]^+$ (53/52), 241/243 $[M - EtO - Br]^+$ (100/100), 227/229 (37/32), 185/187 (23/25). MS (+)-ESI, *m/z*: 387.9153 $[M + Na]^+$. $C_{11}H_{13}{}^{79}Br_2NO_3Na$. Calculated: *m/z* 387.9160.

**Ethyl (*E*-3,5-dibromo-4-ethoxy-1-hydroxy-4-methoxycyclohexa-2,5-dien-1-yl)acetate (20)**, amorphous compound. IR ($CDCl_3$), ν/cm$^{-1}$: 3495 (OH), 1715 (C=O), 1665, 1633 (*cis*-C=C), 1204 (O=C—OEt), 1163, 1102, 1065, 1018 (OMe, OEt). $^1$H and $^{13}$C NMR spectra ($CDCl_3$) of compounds **20**—**22** and **24** are given in Tables 1 and 3, respectively. HMBC ($CDCl_3$, 500 MHz), H/C: 2 (6)/3 (5), 4, 6 (2), 7; 7/1, 2, 6, 8; $OCH_3$/4; $OC\underline{H}_2CH_3$/4, $OCH_2\underline{C}H_3$; $OCH_2C\underline{H}_3/O\underline{C}H_2CH_3$; OH/1, 2, 6, 7; $COOC\underline{H}_2CH_3$/8, $COOCH_2\underline{C}H_3$; $COOCH_2C\underline{H}_3/COO\underline{C}H_2CH_3$. MS (EI, 70 eV), *m/z* ($I_{rel}$ (%)): 380/382/384 $[M - MeOH]^+$ (8/20/12), 366/368/370 $[M - EtOH]^+$ (3/6/3), 325/327/329 $[M - CH_2COOEt]^+$ (34/64/32), 307/309/311 $[M - CH_2COOEt - H_2O]^+$ (16/20/10), 246/248 $[M - CH_2COOEt - Br]^+$ (99/100), 185/187 (16/16). MS (+)-ESI, *m/z*: 434.9418 $[M + Na]^+$. $C_{13}H_{18}{}^{79}Br_2O_5Na$. Calculated: *m/z* 434.9419.

**Ethyl (*Z*-3,5-dibromo-4-ethoxy-1-hydroxy-4-methoxycyclohexa-2,5-dien-1-yl)acetate (21)**, amorphous compound. IR ($CDCl_3$), ν/cm$^{-1}$: 3489 (OH), 2833 (OMe), 1714 (C=O), 1665, 1632 (*cis*-C=C), 1205 (O=C—OEt), 1163, 1103, 1094, 1064, 1019 (OMe, OEt). HMBC ($CDCl_3$, 500 MHz), H/C: 2 (6)/3 (5), 4, 6 (2), 7; 7/1, 2, 6, 8; $OCH_3$/4; $OC\underline{H}_2CH_3$/4, $OCH_2\underline{C}H_3$; $OCH_2C\underline{H}_3/O\underline{C}H_2CH_3$; OH/1, 2, 6, 7; $COOC\underline{H}_2CH_3$/8, $COOCH_2\underline{C}H_3$; $COOCH_2C\underline{H}_3/COO\underline{C}H_2CH_3$. MS (EI, 70 eV), *m/z* ($I_{rel}$ (%)): 380/382/384 $[M - MeOH]^+$ (4/7/3), 366/368/370 $[M - EtOH]^+$ (9/20/9), 325/327/329 $[M - CH_2COOEt]^+$ (25/44/21), 307/309/311 $[M - CH_2COOEt - H_2O]^+$ (10/17/10), 246/248 $[M - CH_2COOEt - Br]^+$ (98/100), 185/187 (18/19). MS (+)-ESI, *m/z*: 434.9421 $[M + Na]^+$. $C_{13}H_{18}{}^{79}Br_2O_5Na$. Calculated: *m/z* 434.9419.

**Ethyl (3,5-dibromo-4,4-diethoxy-1-hydroxycyclohexa-2,5-dien-1-yl)acetate (22)**, amorphous compound. HMBC ($CDCl_3$, 500 MHz), H/C: 2 (6)/3 (5), 4, 6 (2), 7; 7/1, 2, 6, 8; $OC\underline{H}_2CH_3$/4, $OCH_2\underline{C}H_3$; $OCH_2C\underline{H}_3/O\underline{C}H_2CH_3$; $COOC\underline{H}_2CH_3$/8, $COOCH_2\underline{C}H_3$; $COOCH_2C\underline{H}_3/COO\underline{C}H_2CH_3$. MS (+)-ESI, *m/z*: 448.9566 $[M + Na]^+$. $C_{14}H_{20}{}^{79}Br_2O_5Na$. Calculated: *m/z* 434.9575.

**(*E*-3,5-Dibromo-4-ethoxy-1-hydroxy-4-methoxycyclohexa-2,5-dien-1-yl)acetic acid (23) and (*Z*-3,5-dibromo-4-ethoxy-1-hydroxy-4-methoxycyclohexa-2,5-dien-1-yl)acetic acid (24)**, amorphous mixture (~1 : 2). IR ($CDCl_3$), ν/cm$^{-1}$: 3495 (OH), 3300—2400 (CO<u>O</u>H dimers of acids), 2834 (OMe), 1734 (C=O monomer), 1714 (C=O dimer), 1664, 1632 (*cis*-C=C), 1141, 1104, 1096, 1063 (OMe, OEt). $^1$H NMR spectrum ($CDCl_3$) is given in Table 1. HMBC ($CDCl_3$, 500 MHz), H/C: 2 (6)/3 (5), 4, 6 (2), 7; 7/1, 2, 6, 8; $OCH_3$/4; $OC\underline{H}_2CH_3$/4, $OCH_2\underline{C}H_3$; $OCH_2C\underline{H}_3/O\underline{C}H_2CH_3$. MS (EI, 70 eV), *m/z* ($I_{rel}$ (%)): 352/354/356 $[M - MeOH]^+$ (9/16/12), 338/340/342 $[M - EtOH]^+$ (14/21/10), 32 (100). MS (+)-ESI, *m/z*: 406.9108 $[M + Na]^+$. $C_{11}H_{14}{}^{79}Br_2O_5Na$. Calculated: *m/z* 406.9106. MS (–)-ESI, *m/z*: 382.9138 $[M - H]^-$. $C_{11}H_{13}{}^{79}Br_2O_5$. Calculated: *m/z* 382.9130.

Acid **24**, amorphous compound. IR ($CDCl_3$), ν/cm$^{-1}$: 3497 (OH), 3300—2400 (CO<u>O</u>H dimers of acids), 2834 (OMe), 1733 (C=O of monomer), 1713 (C=O of dimer), 1664, 1631 (*cis*-C=C), 1142, 1104, 1063 (OMe, OEt). HMBC ($CDCl_3$, 500 MHz), H/C: 2 (6)/3 (5), 4, 6 (2), 7; 7/1, 2, 6, 8; $OCH_3$/4; $OC\underline{H}_2CH_3$/4, $OCH_2\underline{C}H_3$; $OCH_2C\underline{H}_3/O\underline{C}H_2CH_3$. MS (EI, 70 eV), *m/z* ($I_{rel}$ (%)): 352/354/356 $[M - MeOH]^+$ (9/16/12), 338/340/342 $[M - EtOH]^+$ (14/21/10), 32 (100). MS (+)-ESI, *m/z*: 406.9107 $[M + Na]^+$. $C_{11}H_{14}{}^{79}Br_2O_5Na$. Calculated: *m/z* 406.9106. MS (–)-ESI, *m/z*: 382.9135 $[M - H]^-$. $C_{11}H_{13}{}^{79}Br_2O_5$. Calculated: *m/z* 382.9130.

**[(1*S*,5*S*,6*R*)-3,5-Dibromo-1,6-dihydroxy-4,4-dimethoxycyclohex-2-en-1-yl]acetonitrile (25a)**, amorphous compound. IR ($CDCl_3$), ν/cm$^{-1}$: 3562 (OH), 3467 (OH), 1121, 1046 (OMe). $^1$H NMR spectrum ($CDCl_3$) is given in Table 1. $^{13}$C NMR ($CDCl_3$), δ: 26.3 (C(7)); 48.4 (C(5)); 49.6, 52.3 (2 —O<u>C</u>H$_3$); 73.4 (C(1)); 76.3 (C(6)); 96.9 (C(4)); 124.2 (C(3)); 134.5 (C(2)). HMBC ($CDCl_3$, 500 MHz), H/C: 2/3, 4, 6, 7; 5/3, 4, 6, 1; 7/1, 2; $OCH_3$/4. MS (+)-ESI, *m/z*: 391.9102 $[M + Na]^+$. $C_{10}H_{13}{}^{79}Br_2NO_4Na$. Calculated: *m/z* 391.9109.

**[(1*S*,5*S*,6*R*)-1,6-Diacetoxy-3,5-dibromo-4,4-dimethoxycyclohex-2-en-1-yl]acetonitrile (25c)**, amorphous compound. $^1$H NMR ($CDCl_3$, 700 MHz), δ: 2.06 (s, 3 H, C(1)OAc); 2.22 (s, 3 H, C(6)OAc); 2.875, 2.92 (both d, 1 H each, C(7)H$_2$, *J* = = 16.8 Hz); 3.42, 3.62 (both s, 3 H each, both C(4)OMe); 4.355 (d, 1 H, C(5)H, *J* = 11.3 Hz); 6.07 (d, 1 H, C(6)H, *J* = 11.3 Hz); 6.67 (s, 1 H, C(2)H). $^{13}$C NMR ($CDCl_3$), δ: 20.4 (C(6)OCO<u>C</u>H$_3$); 21.5 (C(1)OCO<u>C</u>H$_3$); 24.2 (C(7)); 51.6 (C(5)); 70.9 (C(6)); 79.2 (C(1)); 97.1 (C(4)); 125.8 (C(3)); 133.6 (C(2)); 168.8 (C(6)O<u>C</u>OCH$_3$); 169.1 (C(1)O<u>C</u>OCH$_3$). HMBC ($CDCl_3$, 700 MHz), H/C: 2/3, 4, 6, 7; 5/4, 6, 1; 6/1, 4, 5, 7, C(6)—O<u>C</u>=O; 7/1, 2, 6; $OCH_3$/4; C(1)—COOC<u>H</u>$_3$/1, C(1)—O<u>C</u>=O; C(6)—COOC<u>H</u>$_3$/6, C(6)—O<u>C</u>=O. MS (+)-ESI, *m/z*: 475.9316 $[M + Na]^+$. $C_{14}H_{17}{}^{79}Br_2NO_6Na$. Calculated: *m/z* 475.9320.

**(3a*S*,7*S*,7a*R*)-5,7-Dibromo-3a-hydroxy-6,6-dimethoxy-3a,6,7,7a-tetrahydro-1-benzofuran-2(3*H*)-one (26)**, amorphous compound. IR ($CDCl_3$), ν/cm$^{-1}$: 1792 (C=O), 1196 (O=C—O), 1120, 1093, 1062, 1035 (OMe). $^1$H NMR spectrum ($CDCl_3$) is given in Table 1. $^{13}$C NMR ($CDCl_3$), δ: 43.0 (C(3)); 46.5 (C(7)); 49.2, 51.5 (2 —O<u>C</u>H$_3$); 74.6 (C(3a)); 87.6 (C(7a)); 96.4 (C(6)); 124.1 (C(5)); 135.6 (C(4)); 171.4 (C(2)). HMBC ($CDCl_3$, 500 MHz), H/C: 3/2, 3a, 4, 7a; 4/5, 6, 7a; 7/3a, 5, 6, 7a; 7a/2, 3a, 4, 6, 7; $OCH_3$/6. MS (EI, 70 eV), *m/z* ($I_{rel}$ (%)): 370/372/374 $[M]^+$ (13/21/11), 339/341/343 $[M - MeO]^+$ (59/100/50), 290/292 $[M - Br]^+$ (45/46), 270/272/274 (43/75/40), 259/261 $[M - MeO - Br]^+$ (22/20), 191/193 (89/68), 44 $[CO_2]^+$ (53).

**(3a*S*,6*R*,7*S*,7a*R*)-5,7-Dibromo-6-ethoxy-3a-hydroxy-6-methoxy-3a,6,7,7a-tetrahydro-1-benzofuran-2(3*H*)-one (27)**, amorphous compound. IR ($CDCl_3$), ν/cm$^{-1}$: 3578 (OH), 1791 (C=O), 1199 (O=C—O), 1123, 1096, 1056, 1037 (OMe). $^1$H NMR spectrum ($CDCl_3$) is given in Table 1. $^{13}$C NMR ($CDCl_3$), δ: 14.8 (—OCH$_2$<u>C</u>H$_3$); 43.0 (C(3)); 46.7 (C(7)); 49.0 (—O<u>C</u>H$_3$); 61.1 (—O<u>C</u>H$_2$CH$_3$); 74.5 (C(3a)); 87.5 (C(7a)); 96.0 (C(6)); 124.4 (C(5)); 135.3 (C(4)); 171.6 (C(2)). HMBC ($CDCl_3$, 500 MHz), H/C: 3/2, 3a, 4, 7a; 4/3, 3a, 5, 6, 7a; 7/3a, 5, 6, 7a; 7a/2, 3a, 4, 6, 7; $OCH_3$/6; $OC\underline{H}_2CH_3$/6, $OCH_2\underline{C}H_3$; $OCH_2C\underline{H}_3/O\underline{C}H_2CH_3$. MS (EI, 70 eV), *m/z* ($I_{rel}$ (%)): 384/386/388 $[M]^+$ (8/15/9), 353/355/357 $[M - MeO]^+$ (8/14/8), 339/341/343 $[M - EtO]^+$ (48/100/51), 304/306 $[M - Br]^+$ (23/30), 284/286/288 (22/46/18), 191/193 (75/66), 44 $[CO_2]^+$ (43), 32 $[MeOH]^+$ (75). MS (+)-ESI, *m/z*: 406.9095 $[M + Na]^+$. $C_{11}H_{14}{}^{79}Br_2O_5Na$. Calculated: *m/z* 406.9106.

**(3a*S*,7*R*,7a*R*)-5,7-Dibromo-3a-hydroxy-6,6-dimethoxy-3a,6,7,7a-tetrahydro-1-benzofuran-2(3*H*)-one (28)**, amorphous compound. IR ($CDCl_3$), ν/cm$^{-1}$: 1797 (C=O), 1122, 1098, 1062 (OMe). $^1$H NMR spectrum ($CDCl_3$) is given in Table 1. $^{13}$C NMR ($CDCl_3$), δ: 42.1 (C(3)); 51.7 (C(7)); 47.9, 51.7 (2 —O<u>C</u>H$_3$);

75.5 (C(3a)); 83.2 (C(7a)); 97.2 (C(6)); 126.0 (C(5)); 132.0 (C(4)); 173.6 (C(2)). HMBC ($CDCl_3$, 500 MHz), H/C: 3/2, 3a, 4, 7a; 4/3, 5, 6, 7a; 7/3a, 5, 6, 7a; 7a/2, 3a, 4, 6, 7; $OCH_3$/6. MS (EI, 70 eV), $m/z$ ($I_{rel}$ (%)): 370/372/374 $[M]^+$ (4/5/3), 339/341/343 $[M - MeO]^+$ (43/90/47), 290/292 $[M - Br]^+$ (15/19), 270/272/274 (16/25/17), 259/261 $[M - MeO - Br]^+$ (21/20), 191/193 (66/54), 44 $[CO_2]^+$ (100).

**Sodium [(1*S*,6*R*)-3,5-dibromo-1,6-dihydroxy-4-methoxycyclohexa-2,4-dien-1-yl]acetate (29)**, amorphous compound, $[\alpha]_D^{25}$ +20 (*c* 0.075, MeOH). UV (MeOH), $\lambda_{max}$/nm: 281. IR (KBr), $\nu/cm^{-1}$: 2853 (=C—O<u>C</u>H$_3$), 1581 ($COO^-$), 1025 (=C—<u>O</u>CH$_3$). $^1$H NMR spectrum (DMSO-$d_6$) is given in Table 1. $^{13}$C NMR (DMSO-$d_6$, 125 MHz), δ: 42.4 (C(7)); 59.2 (C(4)—O<u>C</u>H$_3$); 74.0 (C(1)); 78.0 (C(6)); 113.8 (C(5)); 116.5 (C(3)); 137.0 (C(2)); 146.4 (C(4)); 176.1 (C(8)). HMBC (DMSO-$d_6$, 500 MHz), H/C: 2/1, 3, 4, 6, 7; 6/1, 2, 4, 5, 7; 7/1, 2, 6, 8; $OCH_3$/4. MS (EI, 70 eV), $m/z$ ($I_{rel}$ (%)): 338/340/342 $[M - H_2O]^+$ (21/39/17), 320/322/324 $[M - 2\,H_2O]^+$ (38/80/39), 306/308/310 (7/16/8), 292/294/296 $[M - 2\,H_2O - CO]^+$ (40/79/37), 278/280/282 (10/28/13), 264/266/268 (12/25/12), 221/223/225 (9/17/9), 213/215 $[M - 2\,H_2O - CO - HBr]^+$ (16/14), 44 (61), 32 (100). MS (–)-ESI, $m/z$: 354.8834 $[M]^-$. $C_9H_9O_5Br_2$. Calculated: $m/z$ 354.8822.

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