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Lucibufagins, IV. New defensive steroids and a pterin from the firefly *Photinus pyralis* (Coleoptera: Lampyridae)[1]

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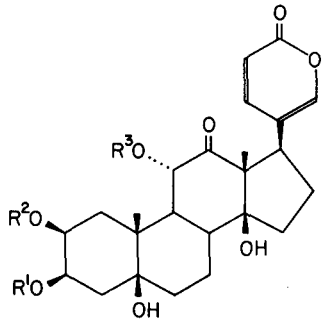
Summary. Two new defensive steroids (lucibufagins **6** and **7**) and a fluorescent pterin (**8**) have been isolated and characterized from the firefly *Photinus pyralis.*

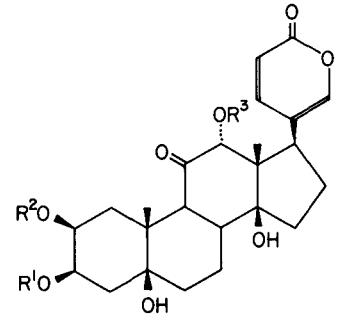
A previous paper[3] from our laboratories described the characterization of the 5 major defensive steroids (**1**–**5**) of the firefly *Photinus pyralis* (Coleoptera: Lampyridae), all esters of 12-oxo-2β, 5β, 11α-trihydroxybufalin (**6**). We now wish to report the completion of this study: two last lucibufagins, **6** and **7**, as well as a pterin **8** have been isolated and identified.

The methanol extract (2.70 g) of 14,000 *P. pyralis* specimens was submitted to Sephadex LH-20 gel filtration (elution with methanol). Bioassay[4] of the resulting fractions showed that **6** and **7** were responsible for the activity of the extract. Column chromatography (silica gel, hexane-tetrahydrofuran 4:1 to 1:9) yielded enriched fractions of **6** and **7**. Whereas purification of **7** (m.p. 182–84 °C) required semi-preparative reversed-phase HPLC (μ-Bondapak C_{18}, methanol-water 2:3, 1.0 ml/min, detection at 313 nm, quantity isolated 3.5 mg), **6** (m.p. 170–72 °C, $[\alpha]_D^{20}+24.5°$, c = 0.014 in methanol) was obtained pure (36 mg) by a 2nd gel filtration. During the latter process, 38 mg of crude **8** were also collected. This final compound was conveniently purified by droplet countercurrent chromatography (DCC-A apparatus, Tokyo Rikakikai Ltd, chloroform-methanol-water 7:13-8 used in the descending mode).

Structural analysis of the steroids was based on the following data: for **6** the molecular formula $C_{24}H_{32}O_8$ was established by high resolution mass spectrometry (M^+ 448.2075, calculated 448.2097); of the 24 carbon signals directly observed in the ^{13}C-NMR-spectrum, 20 were within 0.4 ppm of the analogous signals for **3**[3]; **6** could be acetylated to give the triacetate **9** which corresponded (MS and HPLC) to the 11-acetyl derivative of **3**[3]; finally **6** was found to be identical in all respects (HPLC, TLC, m.p., MS, ^{1}H-NMR) to the deacetylated compound obtained by ammonolysis[5] of **3**. Lucibufagin **7** had the same molecular formula as **6** (M^+ 448.2011); it was also obtained in small amount by prolonged ammonolysis of **3**. Further spectral analysis (EI-MS, ^{1}H-NMR) clearly indicated **7** to be an 11-oxo-12-hydroxy isomer of **6**; the α configuration of the C-12 hydroxyl was apparent from the ^{1}H-NMR-spectrum of its triacetyl derivative **10** (for details see Goetz et al.[5] and Huber et al.[6].

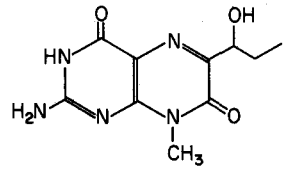


	R^1	R^2	R^3
1	$(CH_3)_2CHCO$	CH_3CO	H
2	CH_3CH_2CO	CH_3CO	H
3	CH_3CO	CH_3CO	H
4	CH_3CO	H	H
5	H	CH_3CO	H
6	H	H	H
9	CH_3CO	CH_3CO	CH_3CO



	R^1	R^2	R^3
7	H	H	H
10	CH_3CO	CH_3CO	CH_3CO

Compound **8** (m.p. > 350 °C; $[a]_D^{20} + 113.1°$, c = 0.011 in methanol; IR (KBr pellet): 3400 - 3300, 2920, 1665, 1640, 1620, 1510, 1450, 1405 cm^{-1}) is strongly fluorescent when viewed under UV-light; its UV-spectrum [λ_{max}^{MeOH} (log ε): pH = 7, 344 (4.17), 295 (3.98), 225 (4.19) nm; pH = 10, 360 (4.18), 278 sh (3.50), 260 (4.04), 220 (4.02) nm] was indicative of a pterin[7]; the molecular formula obtained by high resolution mass spectrometry was $C_{10}H_{13}N_5O_3$ ($M^{+\cdot}$ 251.0657, calculated 251.0663). The EI mass spectrum indicated facile loss of H_2O and C_2H_5. 10 carbon signals were visible in the ^{13}C-NMR-spectrum [80 MHz, DMSO-d_6: δ 175.5 (s), 168.7 (s), 164.9 (s), 160.1 (s), 156.3 (s), 119.8 (s),



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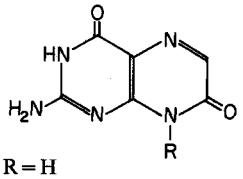
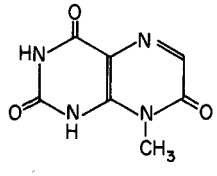


11 R = H
12 R = CH_3



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79.4 (d), 36.9 (q), 36.4 (t), 19.4 (q)]. The ^1H-NMR data [90 MHz, DMSO-d_6; δ 0.90 (3,t), 1.85 (2,m), 3.43 (3,s), 4.72 (2,br; + D_2O: 1,dd), 7.25 (2,br; exchange with D_2O)] indicated a 1-hydroxypropyl side chain and an N-methyl grouping. Compound **8** formed a diacetate (acetic anhydride-pyridine, RT) whose ^1H-NMR spectral characteristics were completely consistent with the structural features described above. We propose structure **8** [6-(1′hydroxypropyl)-8-methylisoxanthopterin] for this pterin on the basis of extensive comparison of its spectral data with those reported[7-9] for several similar compounds. It bears close resemblance to the well known isoxanthopterin **11** and its 8-methyl derivate **12**[7], as well as to luciopterin (**13**), reported to occur in *Luciola cruciata,* a Japanese firefly[10]. It differs quite clearly, however, from the as yet incompletely characterized fluorescent compound 'luciferesceine', isolated from *P. pyralis* by Strehler[11].

The characterization of **6** and **7** completes our study of the distasteful principles of *P. pyralis.* It is interesting to note that **6** is the unesterified parent of lucibufagins **1-5.** Although **6** was consistently detected in the HPLC analysis of a large number of fresh extracts of freeze-dried individual specimens and is most likely a natural component, **7** (which was found in much smaller quantities than **1-6**) may be an artifact arising from isomerization during isolation, especially in view of the fact that 11a-hydroxy-12-oxo-bufadienolides are partially isomerized to their 11-oxo-12a-hydroxy counterparts on alumina[6].

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2 To whom reprint requests should be addressed.
3 J. Meinwald, D. Wiemer and T. Eisner, J. Am. chem. Soc. *101,* 3055 (1979).
4 T. Eisner, D. Wiemer, L. Haynes and J. Meinwald, Proc. natl Acad. Sci. USA *75,* 905 (1978).
5 M. Goetz, D. Wiemer, L. Haynes, J. Meinwald and T. Eisner, Helv. chim. Acta *62,* 1396 (1979).
6 K. Huber, H. Linde and K. Meyer, Helv. chim. Acta *50,* 1994 (1967).
7 W. Pfleiderer and E. Taylor eds., Pteridine Chemistry. Macmillan Company, New York 1964.
8 R. Blakley, The Biochemistry of Folic Acid and Related Pteridines. Elsevier, Amsterdam 1969.
9 A. Dieffenbacher, R. Mondelli and W. von Philipsborn, Helv. chim. Acta *49,* 1355 (1966); A. Dieffenbacher and W. von Philipsborn, Helv. chim. Acta *52,* 743 (1969).
10 Y. Kishi, S. Matsuura, S. Inoue, O. Shimomura and T. Goto, Tetrahedron Lett. *1968,* 2847.
11 B. Strehler, Archs Biochem. Biophys. *32,* 397 (1951).

Bioactive marine metabolites I. Isolation of guaiazulene from the gorgonian *Euplexaura erecta*[1]

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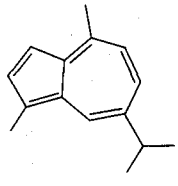
Summary. The isolation and identification of guaiazulene from the gorgonian *Euplexaura erecta* is described.

In recent years sesqui- and diterpenes have been isolated from gorgonians and soft corals[2]. Some of them possess antibacterial, antifungal, antineoplastic, or ichthyotoxic activity[3,4]. In the course of our study on antimicrobial substances from Japanese marine invertebrates we found that the ether soluble material from a methanol extract of the gorgonian *Euplexaura erecta* showed mild activity against *Pseudomonas aeruginosa.* We have isolated from this extract guaiazulene (**1**) as the active substance, which is the subject of this paper.



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Fresh specimens of *E. erecta* (20 g) collected at Enoshima Island, Kanagawa, Japan, were ground and extracted with diethyl ether. The solvent was removed below 30 °C to yield a brownish blue oil which was chromatographed twice on silica gel columns, first with diethyl ether, then with n-hexane. The characteristic blue band was collected as an active fraction to obtain 40 mg of **1**(0.2% wet weight) as a bluish violet oil. It showed a mild activity against fungi, gram-positive and gram-negative bacteria. The high resolution mass spectrum showed the molecular formula $C_{15}H_{18}$ (m/z 198.1452, req. 198.1408). Combustion analysis data