and this further indicates that similar biosynthetic potentials exist in both marine algae and invertebrate animals.

Summary. A sesquiterpene-substituted 4-hydroxybenzoic acid, zonaroic acid (**5**), is described from the brown seaweed *Dictyopteris undulata* (= *zonarioides*). The absolute stereochemistry in the zonarol (**1**)-chromazonarol (**2**) and zonaroic acid (**5**) series has also been defined. The occurrence of **5** along with zonarol (**1**), the corresponding sesquiterpene-substituted hydroquinone, suggests that 4-hydroxybenzoic acid is the ring precursor as in ubiquinone biogenesis.

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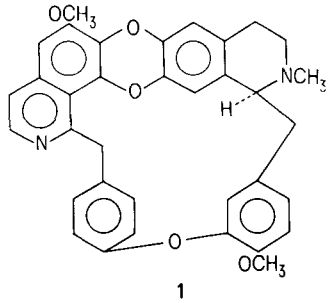
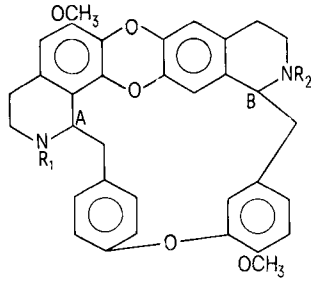
Trigilletimine: A New *Bis*benzylisoquinoline Alkaloid from *Triclisia* Species[1]

In a previous communication[2] the isolation of an unidentified base referred to as TGS-1 from an acidic extract of the stems and roots of *Triclisia gilletii* (De Wild.) Staner and *T. patens* Oliv. was reported, along with other *bis*benzylisoquinoline alkaloids. This paper is to report the structure and stereochemistry of this base which has been named trigilletimine.

Trigilletimine (**1**), crystallized from ethanol as white needles, mp 284° (dec); $[\alpha]_D^{25}$ −285.7° (c 0.7, CH_2Cl_2); λ_{max}^{MeOH} 210 nm (log ε 4.72), 232 (sh) (4.67), 273 (sh) (4.21), 311 (sh) (3.46) and 351 (3.05) with a bathochromic shift in acidic methanol; $\delta_{60\ MHz}^{CDCl_3}$ 2.40 (s) (3H) (NCH_3), 3.92 (s) (3H) (OCH_3), 3.99 (s) (3H) (OCH_3), 5.86–7.29 (m) (1OH) (ArH), 7.39 (d) (1H, J = 6Hz) and 8.34 (d) (1H, J = 6 Hz);



1



2 R_1 = H, R_2 = CH_3; A = R, B = S
3 R_1 = R_2 = CH_3; A = R, B = S
4 R_1 = H, R_2 = CH_3; A = B = S
5 R_1 = CH_3, R_2 = H; A = B = R
6 R_1 = R_2 = CH_3; A = S, B = R
7 R_1 = CH_3, R_2 = H; A = R, B = S
8 R_1 = CH_3, R_2 = H; A = S, B = R

M+ *m/e* 558 (89%) (measured 558.2131 and calculated 558.2154 for $C_{35}H_{30}N_2O_5$), 557 (100), 543 (32), 279 (36), 211 (8), 210.5 (10) and 189 (6). The base gave a positive dibenzodioxin test with a mixture of nitric and sulfuric acids[3].

Catalytic reduction of trigilletimine in ethanol over 5% Pd-C for 12 h afforded tetrahydrotrigilletimine (**2**), mp starts decomp. at 187°; $[\alpha]_D^{17}$ −160° (c 1.0, CH_3OH); $\lambda_{max}^{CH_3OH}$ 211 nm (log ε 4.15), 238 (sh) (4.05), 280 (3.23), 307 (sh) (3.05); $\delta_{60\ MHz}^{CD_3OD}$ 2.88 (s) (3H) (NCH_3), 3.89 (s) (6H) (2 OCH_3), 6.04–7.17 (10H) (ArH); M+ *m/e* 562 (57%) for $C_{35}H_{34}N_2O_5$, 561 (41), 547 (5), 363 (6), 349 (16), 336 (27), 335 (100), 321 (28) and 168 (16).

Treatment of **2** with CH_2O and $NaBH_4$ gave N-methyltetrahydrotrigilletimine (**3**) mp 178–182° dec; $[\alpha]_D^{20}$ −209° (c 0.45, $CHCl_3$); $\lambda_{max}^{CH_3OH}$ 210 nm (log ε 4.10), 235 (sh) (4.01), 275 (sh) (3.54), 288 (sh) (3.49), 345 (sh) (3.00); $\delta_{60\ MHz}^{CDCl_3}$ 2.50 (s) (3H) (NCH_3), 2.56 (s) (3H) (NCH_3), 3.89 (s) (3H) (OCH_3), 3.93 (s) (3H) (OCH_3), and 6.00–7.50 (10H) (ArH); M+ *m/e* 576 (56%) for $C_{36}H_{36}N_2O_5$, 350 (37), 349 (100), 335 (59) and 175 (84).

The NMR-spectrum of trigilletimine showed a pair of doublets characteristic of *ortho* aromatic protons at δ 8.34 (1H, J = 6 Hz) and 7.39 (1H, J = 6 Hz), each of which collapsed to a singlet upon irradiation of the other. The same behavior is exhibited by the C-3 (δ 8.33 [d, 1H, J = Hz]) and C-4 (δ 7.36 [d, 1H, J = 6 Hz]) protons of papaverine. The molecular ion appeared at *m/e* 558 which is 4 mass units less than that of some secondary amino alkaloids of this group such as trilobine[4] (**4**) and *O*-methylmicranthine[5] (**5**). The large M, M-1 and M-15 ions were suggestive of an isoquinoline system similar to papaverine[6]. All of these data, along with the bathochro-

[1] Part XII in the series 'Constituents of West African Medicinal Plants'. For Part XI see D. Dwuma-Badu, J. S. K. Ayim, A. N. Tackie, J. E. Knapp, D. J. Slatkin and P. L. Schiff, jr., Phytochemistry, in press (1975).
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mic shift in acidic medium, suggested that trigilletimine was a benzylisoquinoline-benzyltetrahydroisoquinoline dimeric alkaloid of the dibenzodioxin type containing 2 methoxy and 1 N-methyl groups.

N-methyltetrahydrotrigilletimine (**3**) was shown to be the enantiomer of N-methyltelobine[5] (**6**) (mp 175–180° dec; $[\alpha]_D^{18}$ +248° [$CHCl_3$]) by direct comparison (uv, ir, nmr, ms, mp. $[\alpha]_D$). Since N-methyltetrahydrotrigilletimine (**3**) is the enantiomer of N-methyltelobine (**6**), tetrahydrotrigilletimine must be represented by **2** or **7**. This was further substantiated by the fact that tetrahydrotrigilletimine is not identical to trilobine[4] (**4**), *O*-methylmicranthine[5] (**5**) or telobine[5] (**8**) or their enantiomers by comparison (mp, ir, nmr, $[\alpha]_D$). Since telobine (**8**) is the enantiomer of **7**, and neither are identical to tetrahydrotrigilletimine, this indicates that tetrahydrotrigilletimine must be represented by **2** and therefore trigilletimine by **1**. It is of interest to note the stereospecific reduction of trigilletimine to tetrahydrotigilletimine. A consideration of models reveals that this may be due to a particularly stable conformation which subsequently encourages hydrogenation in a stereospecific manner. To our knowledge, this is the first example of a naturally occurring dibenzodioxin alkaloid containing an aromatized isoquinoline ring (which has only been found in one other *bis*benzylisoquinoline alkaloid, the quaternary base phaeantharine chloride from *Phaeanthus ebracteolatus*[7]).

Summary. The structure of trigilletimine (**1**), a new *bis*benzylisoquinoline dibenzodioxin alkaloid from *Triclisia gilletii* (De Wild.) Staner and *T. patens* Oilv., was determined by spectral means and conversion to the N-methyltetrahydro derivative.

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Changes in Major Liver Constituents Following Hypophysectomy in Goldfish (*Carassius auratus*)[1]

Hypophysectomy in teleosts results in species specific changes in liver glycogen levels. In *Anguilla rostrata* liver glycogen was depleted[2] whereas in *Poecilia latipinna* it was elevated[3]. In the latter species, however, comparisons were made with unoperated and not sham-operated fish, thus leaving unaccounted the stress of operation. *Tilapia mossambica*, on the other hand, showed no change in liver glycogen levels following hypophysectomy[4]. However, hypophysectomy can alter liver size as well as glycogen concentrations as shown in *Fundulus heteroclitis;* glycogen concentrations dropped slightly after pituitary removal, but liver size almost doubled resulting in a net accumulation of glycogen[5]. Again comparisons were made with unoperated rather than sham-operated controls. With this in mind we have examined the quantitative and qualitative changes in major constituents of goldfish (*Carassius auratus*) liver following hypophysectomy.

Materials and methods. Medium size goldfish (4–9 g) were obtained from Hartz Mountain Pet Supplies, Rexdale, Ontario and maintained at 20 ± 0.5 °C for at least 1 month prior to operation. Operated and unoperated fish were held in 20 °C tap water. NaCl was added to the water giving a 0.2% solution to insure against osmoregulatory failure in hypophysectomized fish. The fish were fed Purina trout ration daily except on the day of killing. The methods of hypophysectomy and sham operation were reported earlier[6]. Completeness of hypophysectomy was determined by the colour-loss criterion of JOHANSEN and ROY[7] and the absence of any pituitary remnants under moderate magnification at autopsy.

In the 5th week after operation fish were killed by decapitation and their livers were analyzed immediately for their constituents. Lipid content was estimated by the method of BLIGH and DYER[8]. Glycogen was determined by the method of Lo et al.[9] with slight modification. It was found that the glycogen isolation step effected by precipitation with ice-cold ethanol and centrifugation could be omitted without significant change in yield (Table). Protein was assayed by the method of LOWRY et al.[10] and the biuret method[11]. Addition of biuret reagent to the TCA precipitate of diluted ground liver homogenate resulted in a persistent cloudiness which was

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