SPECIALIA

Synthesis and properties of benzo [d,e]estra-1,3,5(10)-triene-3,17β-diol 17-acetate

P. P. Castelli[1], R. Vitali[2], G. Briziarelli[3] and R. Gardi

Vister Research Laboratories, I-22064 Casatenovo (Como, Italy), 28 March 1980

Summary. A new pentacyclic analogue of estradiol, benzo[d,e]estradiol 17-acetate, has been prepared and investigated for its hormonal and antitumor activity. Unlike related testosterone derivatives, the new compound did not display any interesting property.

Some years ago we reported the synthesis of 3-ketobenzo[d,e] steroids from the propargyl enol ethers of the corresponding Δ^4-3-ketosteroids, through a Claisen-like rearrangement and the subsequent cyclodehydrogenation of 4-allylidene ketones[4–6]. Pentacyclic steroids of this kind derived from testosterone proved to be endowed with high antitumor activity and little androgenic activity[5,7]. Here we wish to report on the synthesis and properties of related derivatives in the series of 1,3,5(10)-estratriene.

Reaction of benzo[d,e]estr-4-ene,3,17-dione[4] (**I**) with trimethylorthoformate in the presence of p. toluene-sulfonic acid gave 3,17,17-trimethoxybenzo[d,e]estra-2,4-diene (**II**, m.p. 146–148 °C[8], $[\alpha]_D + 73°$; λ_{max} nm(ε) 222 (12,790), 227 (14,370), 270 (6,670); ν_{max} 1642, 1583, 781, 739 cm^{-1}. Anal. calculated for $C_{24}H_{32}O_3$: C, 78.22; H, 8.75; found: C, 78.05; H, 8.49). Treatment of **II** with N-bromosuccinimide in buffered, aqueous acetone[9,10] and equilibration with HCl afforded 2α-bromobenzo[d,e]estr-4-ene-3,17-dione (**III**, m.p. 204–205 °C (dec.), $[\alpha]_D - 18°$; λ_{max} nm (ε) 270 (10,500), 309 (2,250); ν_{max} 1720, 1672, 1578, 820, 795, 763 cm^{-1}. Anal. calculated for $C_{21}H_{23}O_2Br$: C, 65.12; H, 5.98; Br, 20.64; found: C, 64.96; H, 5.90; Br, 20.29), which by dehydrohalogenation with LiCl in dimethylformamide[11] gave benzo[d,e]estrone[12] (**IV**, m.p. 285 °C (dec.), $[\alpha]_D + 59°$; λ_{max} nm (ε) 222–223 (28,340), 237 (27,330), 305 (7,500), 331 (4,780); λ_{max} 3280, 1718, 1619, 1589, 1518, 822, 806, 795, 776 cm^{-1}. Anal. calculated for $C_{21}H_{22}O_2$: C, 82.32; H, 7.24; found: C, 82.03; H, 6.94).

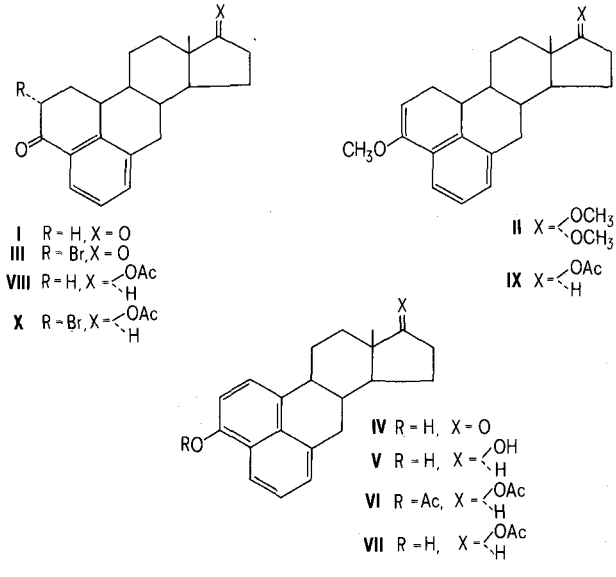
Conventional $NaBH_4$ reduction of **IV** afforded benzo[d,e]estra-1,3,5(10)-triene-3,17β-diol (benzo[d,e]estradiol, **V**, m.p. 260–262 °C, $[\alpha]_D - 29.5°$; λ_{max} nm (ε) 219 (29,570), 236 (27,430), 303 (7,440), 332 (4,725); ν_{max} 3550, 3210, 1614, 1585, 1512, 828, 811, 799, 785, 761 cm^{-1}. Anal. calculated for $C_{21}H_{24}O_2$: C, 81.78; H, 7.84; found: C, 81.70; H, 7.74), then acetylated to the diacetate **VI**, m.p. 203–205 °C, $[\alpha]_D - 45°$; λ_{max} nm (ε) 227 (69,200), 289 (9,100); ν_{max} 1750, 1734, 1614, 1595, 1506, 1242, 1198, 809, 802, 782, 768 cm^{-1}. Anal. calculated for $C_{25}H_{28}O_4$: C, 76.50; H, 7.19; found: C, 76.35; H, 7.25. Selective hydrolysis of **VI** with K_2CO_3 in methanol gave rise to benzo[d,e]estra-1,3,5(10)-triene-3,17β-diol 17-acetate (**VII**, m.p. 285 °C (dec.), $[\alpha]_D - 50°$; λ_{max} nm (ε) 222–223 (28,750), 237 (27,150), 304–306 (7,200); ν_{max} 3420, 1702, 1618, 1593, 1518, 1278, 826, 804, 793, 773 cm^{-1}. Anal. calculated for $C_{23}H_{26}O_3$: C, 78.82; H, 7.48: found: C, 79.05; H, 7.45).

Alternatively, benzo[d,e]estradiol 17-acetate (**VII**) was prepared from the known 17β-acetoxy-benzo[d,e]estr-4-en-3-one (**VIII**)[13], according to the same procedure. 3-Methoxybenzo[d,e]estra-2,4-dien-17β-ol acetate (**IX**, m.p. 229–231 °C, $[\alpha]_D + 53°$; λ_{max} nm (ε) 227 (14,300); 268 (6,760); ν_{max} 1718, 1641, 1582, 1253, 787, 739 cm^{-1}. Anal. calculated for $C_{24}H_{30}O_3$: C, 78.65; H, 8.25; found: C, 78.34; H, 8.19), prepared from **VIII**, was brominated with NBS to give 2α-bromo-17β-acetoxybenzo[d,e]estr-4-en-3-one (**X**, m.p. 203–205 °C, $[\alpha]_D - 78.5°$; ν_{max} 1728, 1678, 1584, 1245, 826, 796, 763 cm^{-1}. Anal. calculated for $C_{23}H_{27}O_3Br$: C, 64.03; H, 6.31; Br, 18.53; found: C, 64.34; H, 6.31; Br, 18.22). Dehydrobromination of the latter gave **VII**.

Benzo[d,e]estradiol 17-acetate (**VII**) was investigated in several tests for its hormonal and antihormonal activity. After s.c. administration in rats, it did not display any significant uterotrophic, antiuterotrophic, androgenic, antiandrogenic or antifertility activity at doses as high as 10 mg total, 20 mg total, 8.75 mg daily, 10 mg total, and 2 mg daily, respectively. In the parabiosis test[14], **VII** exhibited a significant antigonadotrophic activity only at the daily dose of 3.5 mg.

Tested in rats bearing hormone-dependent mammary tumors induced by 7,12-dimethylbenz[a]anthracene[15], it displayed no effect at daily doses up to 1 mg for 30 days.

We may conclude that the modification of estradiol molecule by insertion of the additional aromatic ring in d,e-position eliminates almost completely the hormonal prop-



erties of the parent compound, without inducing the interesting dissociation of activities displayed by the testosterone analogues[5,7].

1 Present address: Blasinachim S.p.A., Milano, Italy.
2 Present address: Farnex Laboratori S.p.A., Codogno, Italy.
3 Present address: Schering Corporation, Lafayette, New Jersey, USA.
4 R. Gardi, R. Vitali and P.P. Castelli, Tetrahedron Lett. *27*, 3203 (1966).
5 G. Briziarelli, P.P. Castelli, R. Vitali and R. Gardi, Experientia *29*, 618 (1973).
6 For an alternate synthesis see T. Komeno, S. Ishihara, K. Takigawa, H. Itani and H. Iwakura, Chem. Pharm. Bull *17*, 2586 (1969).
7 G. Briziarelli, Endocrinology *81*, 390 (1967).
8 Melting points are uncorrected. Optical rotations were taken in 0.5% dioxane solutions at 24± 1°. UV-spectra were determined in 95% EtOH and IR-spectra in Nujol. We are indebted to Dr C. Pedrali for the spectral determinations.
9 H.J. Ringold, E. Batres, A. Bowers, J. Edwards and J. Zderic, J. Am. chem. Soc. *81*, 3485 (1959).
10 R. Gardi, P.P. Castelli and A. Ercoli, Tetrahedron Lett. *23*, 497 (1962).
11 R.P. Holysz, J. Am. chem. Soc. *75*, 4432 (1953).
12 Systematic name: 2,3a,4,5,5a,12,12a,12b-octahydro-8-hydroxy-3aβ-methylbenzo[f,g]cyclopent[a]anthracen-3(1H)-one.
13 A. Ercoli and R. Gardi, U.S. patent No. 3, 419, 582 (December 31, 1968).
14 R. Hertz and R.K. Meyer, Endocrinology *21*, 756 (1937).
15 C. Huggins, L.C. Grand and F.P. Brillantes, Nature *189*, 204 (1961).

Termite soldier chemotaxonomy. A new diterpene from the Malaysian nasute termite *Bulbitermes singaporensis*[1]

G.D. Prestwich[2], S.H. Goh and Y.P. Tho

Department of Chemistry, State University of New York, Stony Brook (New York 11794, USA), University Malaya, Kuala Lumpur (Malaysia), and Forest Research Institute, Kepong (Malaysia), 28 March 1980

Summary. The defense secretion of the nasute termite *Bulbitermes singaporensis* consists of 1 new and 2 known tetracyclic diterpenes, spectrometrically identified as 14α-acetoxy-6,8-kempadien-3-one, 3α,14α- and 3β,14α-diacetoxy-6,8-kempadiene. The presence of these compounds supports the kinship of the oriental 'constricted-head' genera with *Nasutitermes* species in the Philippines and in East Africa.

Nasute termite soldiers (Isoptera: Termitidae: Nasutitermitinae) eject an irritating, viscous defense secretion when provoked. Progress has been reported in the elucidation of structures[3] of the mono- and diterpenoid constituents, instances of inter- and intraspecific variation[1,4] and the use of the secretion in defense[1,5]. Recently, we have analyzed defense secretions of nasute genera occupying intermediate phylogenetic positions in the hope of clarifying the evolution of diterpene biosynthesis in this advanced termite subfamily[1,6]. In this paper we describe the diterpenes of an oriental (Malaysian) nasute in the genus *Bulbitermes*, biogeographically and morphologically related to the Oriental-Ethiopian 'constricted-head' genera[7] including *Grallatotermes*[6]. A chemical connection to *Nasutitermes luzonicus*[1,3] (Philippines) and *Nasutitermes kempae*[1,3,8] (East Africa) is thereby established.

The crude defense secretion (15 mg) was obtained by hexane extraction of 1000 soldier heads of *Bulbitermes singaporensis* collected from a single spherical, hard carton arboreal nest in the Lesong Forest Reserve, Pahang, Malaysia. The secretion contained 2.9 µg monoterpene hydrocarbons per soldier (0.5% fresh weight %, which was predominantly α-pinene (89%) and β-pinene (7%) as established by GC-MS. Diterpenes (figure 1) comprised 1.6% fresh weight % of soldiers (10.6 µg/soldier). Chromatography of the crude secretion (Florisil, 10% ethyl acetate-hexane) gave 2 TLC-homogeneous (Polygram Sil UV, R_f 0.20 and 0.27 for 25% ethyl acetate-hexane) materials of 6 mg each. GLC (3% OV-17, 2 mm × 2 m glass column, T_i = 210 °C, T_p = 6 °C min^{-1}, T_f = 270 °C) examination of these materials showed the higher R_f spot (**I**) to be homogeneous; however, the lower spot was a 1:1 mixture of 2 closely-eluting compounds **II** and **III**. Analysis by GC/MS[9] indicated that compound **I** had a parent peak at m/z 342 and a base peak at 282 ($M^+ - HOAc$), and fragmented in an identical manner to kempene-2 (14α-acetoxy-6,8-kempadien-3-one) from *Nasutitermes kempae*[8]. This assignment was confirmed by TLC and GLC coelution and by the identity of the ^{1}H-NMR spectra of these 2 samples[10].

Compounds **II** and **III** gave virtually identical mass spectra which were consistent with that obtained for kempene-1[8]: m/z 386 (1%, M^+), 326 (18%, $M^+ - CH_3CO_2H$), 251 (100%, $M^+ - 2\ CH_3CO_2H - CH_3$). The 2nd peak **III** coeluted with kempene-1; however, the stereochemistry at C-3 had not been assigned in the original paper[8]. We suspected that **II** and **III** were epimeric at 1 of the 2 acetate centers; this was then determined by 2 independent methods as described below.

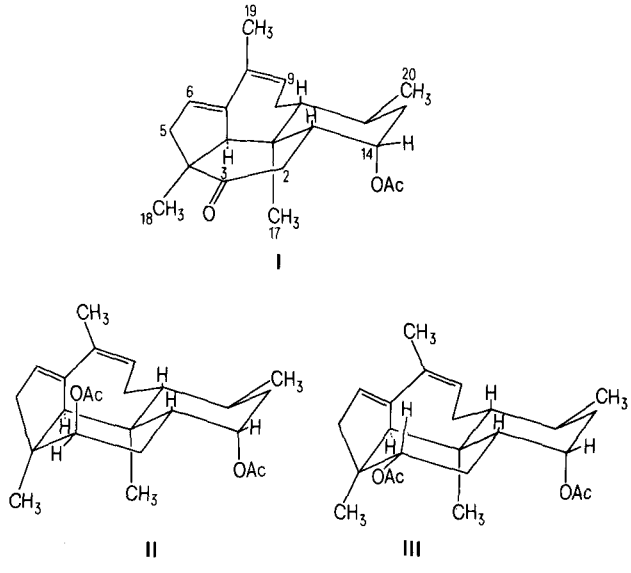


Fig. 1. Stereostructures of diterpenes from *Bulbitermes singaporensis*.