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THE STRUCTURE AND THE CHEMISTRY OF SICCANIN[1] AND RELATED COMPOUNDS

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Abstract—Chemical and spectral properties of the antibiotic, siccanin (I), siccanochromene-E (V), and several of their transformation products are described.

SICCANIN was first isolated by K. Ishibashi *et al.*[2] during their general survey of the constituents of plant pathogenic fungi having antifungal and antibacterial activities. This substance. isolated from *Helminthosporium siccans* Drechsler. which is a parasitic organism of rye-grass. *Lothium multifolium* Lam. exhibits marked activity against *Trichophyton interdigitale* and *T. asteroids*. Clinical tests by topical application proved the effectiveness of siccanin against superficial fungal infections. It was assigned the empirical formula. $C_{22}H_{23}O_4$.[2] however. repetition of the analysis by mass spectrometry gave data consistent with the formula. $C_{22}H_{30}O_3$. The structure including the stereochemistry and the absolute configuration was determined conclusively by a three dimensional X-ray diffraction study of the siccanin *p*-bromobenzenesulfonyl ester.[3] The present paper details the chemistry and spectral properties of siccanin and the congener. siccanochromene-E and of their transformation products.

Siccanin (I). $C_{22}H_{30}O_3$. M^+ at *m/e* 342. m.p. 138°. $[\alpha]_D^{16} = -150°$ (c = 7·75. $CHCl_3$). λ_{max} 278 and 285 nm (ε. 1.640 and 1.800 respectively). NMR signals at δ 0·80 and 0·84 (6H, gem-Me's), 1·25 (3H, s, Me on carbon bearing oxygen), 2·20 (3H, s, ar. Me), 6·15 and 6·30 (2H, s, ar. H's), 3·46 and 4·24 (2H, AB quartet, J = 8 Hz, $-CH_2-O-$), 5·16 (1H, d, J = 8 Hz, benzylic methine), 1·94 (1H, *d*, J = 8 Hz), and 6·57 (1H, s, OH). The doublet at δ 1·94 is decoupled by irradiation at δ 5·16. The mass spectrum of I shows the base peak at *m/e* 175 attributable to the fragment A. Acetylation of I afforded an acetate II. $C_{24}H_{32}O_4$ (M^+, 384) and esterification with *p*-bromobenzenesulfonyl chloride gave crystalline sulfonyl ester III, mp 156°. $C_{28}H_{33}O_5SBr$, which was used for X-ray diffraction studies.

Treatment of siccanin (I) with $BF_3.Et_2O$ in Ac_2O gave two products. IV and VI in a 1:3 ratio. The major product IV. $C_{28}H_{38}O_7$ (M^+. 486) exhibits three Ac signals at δ 1·95. 1·98 and 2·14. and methylene protons of primary acetate at 4·21 and 4·55 (AB quartet, J = 12 Hz). A benzylic proton signal appears at 5·88 as a singlet which indicates that an AcO group at benzylic position possesses α-orientation. since the dihedral angle between C_9 and C_{11} protons in this configuration is about 90°. Hydrolysis of the triacetate IV with alkali gave siccanin (I) by intramolecular participation

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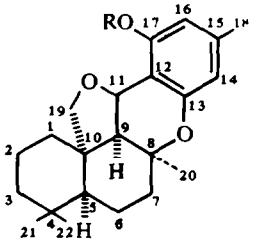
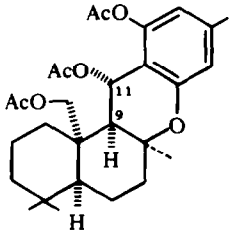
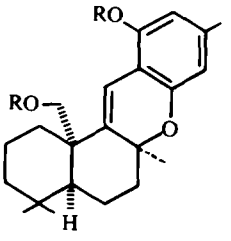
of the primary alcohol in the solvolysis of the benzyl acetate. The second product VI. $C_{26}H_{34}O_5$ (M^+. 426. base peak. 217). λ_{max} 227, 276. 281. 286 and 312 nm (ε. 24.000. 8.440. 7.670. 6.820 and 4.900. respectively). exhibits NMR signals at 0·97. 1·04. 1·32 and 2·24 (four Me's), 3·83, 4·02 (2H, AB quartet, J = 11 Hz, —CH_2OAc), 6·16 (1H, s, olefinic proton). 6·36 and 6·47 (2H. s. ar. H's) and 1·95. 2·28 (6H. s. acetyl Me's). It was proved that this product was identical with the diacetate of siccanochromene-E[4]. which was isolated from the cultured broth of *Helminthosporium siccans.*



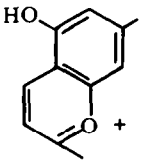
I : R = H
II : R = COMe
III : R = $-SO_2C_6H_4$(p)Br
XVI: R = Me



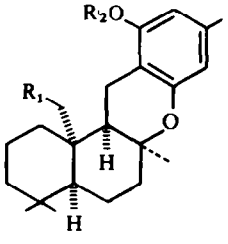
IV



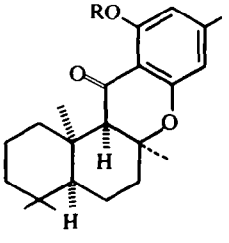
V : R = H
VI: R = COMe



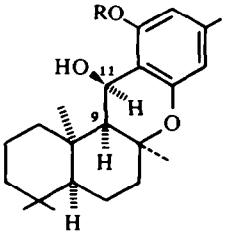
fragment A
M^+ 175
($C_{11}H_{11}O_2$)



VII : R_1 = OH. R_2 = H
VIII: R_1 = OTs. R_2 = Ts
IX : R_1 = H. R_2 = H
X : R = H. R_2 = COMe



XI : R = COMe
XIV: R = Me
XV : R = H



XII : R = H
XIII: R = Me

Siccanochromene-E (V). $C_{22}H_{30}O_3$. mp 191–192°. $[\alpha]_D^{20}$ −86° (EtOH). isolated from *Helminthosporium siccans* as a minor metabolite. shows UV absorption maxima at 234. 287. and 293 nm (ε. 2.6000. 13.900 and 14.100). IR bands at 1.628. 1.580 cm^{-1} and NMR signals at 0·94. 1·01. 1·39 and 2·18 (four Me's). 3·25 and 3·80 (2H. AB quartet. J = 11 Hz). 6·21 and 6·25 (2H. s. ar H's) and 6·59 (1H. s. olefinic proton). The mass spectrum shows the peaks at *m/e* 342 (M^+). 327 (M-15). 311 (M-31) and 175 (base peak). In the NMR spectrum of the siccanochromene-E diacetate. an olefinic proton signal showed marked diamagnetic shift (43 Hz) as compared with that of parent diol V. which indicated the presence of a OH group at C_{17}.[5] These chemical correlations of naturally occuring chromene with siccanin confirms its structure and stereochemistry.

The ether linkage at the benzylic position was also cleaved by the treatment of siccanin with $LiAlH_4$–$AlCl_3$ complex[6] in THF at reflux to afford the diol VII.

$C_{22}H_{32}O_3$ (M^+. 344). NMR δ 2·68 (2H. benzylic protons). 3·62 and 4·04 (2H. AB quartet. J = 13 Hz). The ditosylate VIII. prepared from VII with excess TsCl in pyridine at 85°. was converted into compound IX by $LiAlH_4$ reduction in THF at reflux. In the NMR spectrum of IX. a newly generated Me signal appeared at δ 1·12. Oxidation of the acetate X with chromic acid in aqueous AcOH yielded the ketone XI. $C_{24}H_{32}O_4$ (M^+. 384): λ_{max}223. 265 and 330 nm (19.300. 7.800 and 1.200). ν_{max}1771. 1,680, 1,630 and 1,568 cm^{-1}; NMR 1·82 (1H, s, C_9–H). LAH reduction of the ketone XI gave the benzyl alcohol XII exclusively. In the NMR spectrum of XII. a signal due to a benzylic proton appeared at 5·48 as a doublet. J = 8 Hz. From the coupling constant which is equal to that of siccanin. the configuration of this OH group is assigned the α-orientation (dihedral angle between C_9–H and C_{11}–H is about 45°) and the hydride attack occurred from the less hindered side of the molecule. An attempt was made to convert benzyl alcohol XII into siccanin by irradiation of the ether XIII in the presence of lead tetraacetate and iodine in benzene.[7] however. only the oxidation product XIV was obtained.

EXPERIMENTAL

All m.ps were uncorrected. IR spectra were taken on a Jasco DS-301 spectrophotometer in $CHCl_3$. UV spectra were measured on a Shimadzu SV-50 spectrometer in EtOH. NMR spectra were determined on a Jeol 100 Mc spectrometer using TMS as an internal standard in $CDCl_3$ unless otherwise stated. Mass Spectra were measured on a Hitachi Mass Spectrometer RMU-6D. Merck "Kieselgel G" was used for TLC on 0·25 mm layers. Spots were detected by charring with H_2SO_4 or colour reactions with diazotized benzidine salt solution.

Siccanin (I) Siccanin was obtained as previously described method by K. Ishibashi.[2] The material used was crystallized from MeOH. m.p. 138° ν_{max}3.480 (OH). 1.633. 1.576 (aromatic). 1.350. 1.360 (gem-dimethyls). 1.175 (phenolic C—O). 830 cm^{-1} (isolated aromatic H). (Calc. for $C_{22}H_{30}O_3$: C. 77·15: H. 8·83: 0. 14·02. Found: C. 77·16: H. 8·67: O. 14·16%).

Siccanin acetate (II). Oil. TLC: R_f = 0·55 (benzene:acetone = 20:1). ν_{max} 1.758. 1.630. 1.575. and 870 cm^{-1}. NMR (CCl_4): 0·84 (6H. gem-dimethyls). 1·24 (C_{20}-Me). 2·19 (OCOMe). 2·26 (C_{18}-Me). 1·80 (C_9-H. d. J = 9 Hz). 4·90 (C_{11}-H. d. J = 9 Hz). 3·25 (1H. d. J = 7·5 Hz). 4·05 (1H. d. J = 7·5 Hz). 6·35 (2H. ar. H's).

Siccanin brosylate (III). To a solution of 50 mg of *siccanin* in 1 ml pyridine was added 220 mg of *p*-$BrC_6H_4SO_2Cl$ and the whole was kept at standing at room temp for 18 hr. The mixture was diluted with water and the resulting crystals (70 mg) were recrystallized from MeOH and n-hexane. M.p. 156°. λ_{max}234 (ε. 23.500). 278 (ε. 2.630). 285 nm. ν_{max}1628. 1572 (arom.) 1.375. 1.175 cm^{-1} (=SO_2). NMR: newly appeared A_2B_2-pattern at 7·64 and 7·84 (almost quartet). (Calc. for $C_{28}H_{33}SO_5Br$: C. 59·89: H. 5·92: O. 14·05: S. 5·71: Br. 14·23. Found: C. 59·99: H. 5·99: O. 13·72: S. 6·26; Br. 14·59%).

Siccanin methyl ether (XVI). *Siccanin* (100 mg) was dissolved in EtOH (4 ml) and 0·35 ml of aq. NaOH (NaOH (10 g) in 15 ml of H_2O). To this solution was added 0·5 ml of dimethyl sulfate. and a similar amount of dimethyl sulfate and alkali was added three times. After two hr. the mixture was poured into ice-water and the product isolated with ether. The ethereal extract was washed with water and dried (Na_2SO_4). Evaporation of solvent gave XVI. TLC: R_f = 0·55 (benzene). NMR: 0·80 (6H. gem-dimethyls). 1·16 (C_{20}-Me), 2·23 (C_{18}-CH). 3·80 (OMe), 1·85 (C_9-H, d, J = 8·5 Hz), 4·95 (C_{11}-H, d, J = 8·5 Hz), 3·25, 4·02 (C_{19}-H_2. AB quartet. J = 8 Hz).

Siccanochromene-E *diacetate* (VI) *and compound*-IV. To a solution of 500 mg of *siccanin* in 7 ml of Ac_2O was added a trace amount of BF_3-etherate with stirring at 0°. Deep blue colour appeared and then disappeared (about one min). The mixture was poured into ice-water and extracted with ether. The ethereal extract was washed with 5% aq. $NaHCO_3$. 5% HCl and water and dried (Na_2SO_4). Ether was evaporated to afford 500 mg of residue, which was chromatographed on silica gel (5 g), n-Hexane:benzene = 1:1 fraction gave VI (120 mg) and benzene fraction gave *compd*-IV (300 mg) respectively. VI: glass. λ_{max} 1.760. 1.735. 1.630 and 1.570 cm^{-1}. *Compd*-IV: glass. λ_{max} 226 (sh.). 278 (sh.) and 286 nm (ε. 3700). ν_{max} 1.770. 1.735. 1.635 and

1.586 cm^{-1}. NMR: 0·89. 1·01 (6H. gem-dimethyls). 1·29 (C_{20}–Me). 1·95. 1·98 and 2·14 (three acetyls). 2·28 (C_{18}–CH). 4·21 and 4·55 (2H. AB quartet. C_{19}–H_2. J = 12 Hz). 5·88 (C_{11}–H). 6·38 and 6·48 (2H. ar H's). M^+ at *m/e* 486 ($C_{28}H_{38}O_7$). base peak at *m/e* 175. V gave siccanin on treatment with 5% aq. ethanolic KOH.

Siccanochromene-E (V). Natural *siccanochromene*-E was obtained from the nonsaponifiable fraction of the fermented broth of *H. siccans* after repeated silica gel or florisil column chromatography. Chemically derived *siccanochromene*-E was obtained by the hydrolysis of *siccanochromene*-E diacetate. which was obtained as mentioned above. Identification was confirmed in all respects.

Diol VII. LAH–$AlCl_3$ (1:2) complex was prepared from 80·1 g of $AlCl_3$ and 1·14 g of LAH in 150 ml THF by the method of Brown and Sommerfield.[6] To this solution was added 5·1 g *siccanin* and the whole was refluxed for 22 hr. THF was distilled off under red, press, and ether added. The ethereal solution was washed with water and dried (Na_2SO_4). The extract was chromatographed on silica gel (55 g). Benzene: ether = 6:1 ~ 4:1 fractions gave VII (2 g). NMR: 0·94, 1·10 (6H, gem-dimethyls), 1·19 (C_{20}–Me), 2·16 (C_{18}–Me), *ca.* 2·68 (2H), 3·62 and 4·04 (2H, AB quartet. J = 13 Hz), 6·08 and 6·10 (ar H's). M^+ at *m/e* 344 ($C_{22}H_{32}O_3$).

Ditosylate VIII. To a solution of 1·4 g of *diol* (VII) in 14 ml of pyridine was added 3·4 g of *p*-TsCl and the whole was heated at 85° for three hr and then poured onto ice-water and the product extracted with ether. The ether extract was washed with 5% aq. HCl. 2% aq. NaOH and water and dried (Na_2SO_4). M.p. 202–203° NMR: 0·90. 0·98 (6H. gem-dimethyls). 1·07 (C_{20}–Me). 2·14 (C_{18}–Me). ~ 2·48 (2H. benzylic protons). 2·48 and 2·45 (tosyl Me's). 3·60 and 4·36 (2H. AB quartet. J = 11 Hz. C_{19}–H_2). 6·35 and 6·21 (2H. ar. H's). 7·3–8·0 (8H. Ts.) (Calc. for $C_{36}H_{44}O_7S_2$: C. 66·26: H. 6·75: O. 17·16: S. 9·83. Found: C. 65·97: H. 6·87: O. 17·20: S. 9·96%).

Phenol IX. To a solution of 2·8 g of *di-tosylate* VIII in 140 ml ab. THF was added 4 g LAH and mixture was stirred at reflux for 8 hr. Most of THF was removed under red. press. and then the moist ether added with cooling. The ether extract was washed with water and dried (Na_2SO_4). The crude product was chromatographed on silica gel (23 g). Elution with n-hexane:benzene = 3·1 ~ 1:1 gave the *phenol* IX (1 g). glass ν_{max} 3.620. 3.400. 1.630 and 1.690 cm^{-1}. NMR: 1·37. 1·12 (6H. gem-dimethyls) 1·12 (C_{20}– and C_{19}–Me). 2·09 (C_{18}–Me). 2·3–2·9 (C_{11}–CH_2). 5·43 (OH) 6·0 and 6·12 (2H. ar H's). M^+ at *m/e* 328 ($C_{22}H_{32}O_2$).

Acetate X. IX was acetylated as usual to give *acetate* X. TLC: R_f = 0·7 (benzene:aceton = 20;1). ν_{max} 1.760 (CO). 1.635. 1.582 cm^{-1}. λ_{max} 275 (sh). 283 (ε. 1.700). NMR: 0·90. 1·13 (6H. gem-dimethyls). 1·13 (C_{19}– and C_{20}–Me). 2·20 and 2·21 (C_{18}–Me and acetyl). 6·36 and 6·26 (2H. arom.). M^+ at *m/e* 370 ($C_{24}H_{34}O_3$).

Keto-acetate XI. To an AcOH (8 ml) solution of the *acetate* X (600 mg) was added 2·5 ml of 25% CrO_3 in 80% AcOH. The whole was stood at 24° for 15 hr. MeOH was added and the ethereal extract afforded 530 mg crude XI. purified through silica gel chromatography (ten times). Elution with n-hexane:benzene = 4:1 ~ 1:1 gave *keto-acetate* XI (460 mg). 70% yield. TLC: R_f = 0·58 (benzene:acetone = 12:1). λ_{max} 223 (ε. 19.300). 265 (7.800) and 330 nm (1.200). ν_{max} 1.771. 1.680. 1.630 and 1.568 cm^{-1}. NMR: 0·91. 1·15 (6H. gem-dimethyls) 1·20 (C_{19}– and C_{20}–Me). 2·27 and 2·23 (acetyl and ar. Me's). 1·82 (1H. s. C_9–H). 6·33 and 6·56 (2H. ar. H's). M^+ at *m/e* 384 ($C_{24}H_{32}O_4$).

Keto-phenol XV. XV was obtained by hydrolysis (5% aq. KOH–EtOH) of the *keto-acetate* XI. M.p. 146° (recrystallized from acetone). λ_{max} 214. 285 and 357 nm (22.400. 12.900 and 2.100 respectively). ν_{max} 1.643. 1,625, 1,572, 1,096 and 900 cm^{-1}. NMR: 0·93, 1·18 (6H, gem-dimethyls), 1·23 and 1·33 (C_{19}– and C_{20}–Me), 2·25 (C_{18}–Me). 1·95 (1H. s. C_9–H). 6·06 and 6·17 (2H. arom.). 11·84 (1H. hydrogen-bonding). M^+ at *m/e* 342 ($C_{22}H_{30}O_3$). Calc. for $C_{22}H_{30}O_3$: C. 77·15: H. 8·83. Found: C. 77·29: H. 8·83%).

Keto methyl ether XIV. *Keto-phenol* XV (126 mg) was dissolved in 0·5 ml of aq. NaOH (NaOH 10 g in 15 ml H_2O) solution and 9·5 ml of EtOH. To this was added dimethylsulfate (0·7 ml) with stirring. Same amount of dimethyl sulfate and alkali was added three times. After two hr. the mixture was poured into ice-water and the product isolated with ether. The ethereal extract was washed with 5% aq. NaOH and water and dried (Na_2SO_4). Evaporation of ether afforded XIV (132 mg). TLC: R_f = 0·60 (benzene:acetone = 12:1). NMR: 0·92. 1·16 (6H. gem-dimethyls). 1·20 (C_{19}–Me). 1·25 (C_{20}–Me). 2·28 (C_{18}–Me). 3·82 (—O—Me). 1·82 (1H. s. C_9–H). 6·17 and 6·20 (2H. ar H's). M^+ at *m/e* 356 ($C_{23}H_{32}O_3$).

Carbinol XIII. *Keto methyl ether* XIV (132 mg) was dissolved in abs. ether and to this solution was added 30 mg of LAH slowly. Moist ether was then added and extracted with ether three times. Working up as usual gave 133 mg of crude *carbinol* XIII. Elution with benzene:n-hexane = 5:95 ~ 1:9 on silica gel (3 g) chromatography gave 78 mg of pure *carbinol* XIII (oil). TLC: R_f = 0·3 (benzene:aceton = 12:1). NMR: 0·87. 1·13 and 1·16 (three Me's). 1·45 (C_{20}–Me). 2·25 (C_{18}–Me). 3·87 (–OMe). 1·78 (1H. d. J = 8 Hz). 5·48 (1H. d. J = 8 Hz). (Calc. for $C_{23}H_{34}O_3$. C. 77·05; H. 9·56. Found: C. 77·00: H. 9·60%).

Attempts to convertion of XIII *into siccanin methyl ether* (XVI). (a) The benzene (1·0 ml) solution of *carbinol*

XIII (10 mg) and 10 mg of Pb $(OAc)_4$ was refluxed for 15 hr. Removal of solvent gave the *keto methyl ether* XIV. (b) The benzene (2 ml) solution of *carbinol* XIII (20 mg) and 10 mg of $Pb(OAc)_4$ and I_2 (trace) was irradiated by 300-W Tungsten-lamp under reflux for 5 hr. Removal of solvent gave the *keto methyl ether* XIV exclusively.

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