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THE STRUCTURE OF AGAROTETROL, A NOVEL HIGHLY OXYGENATED CHROMONE FROM AGARWOOD(*JINKO*)

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Fungus infected agarwood(*Aquilaria agallocha* Roxb.) which is distributed in the north eastern district of India is known in the Orient as one of the most prized incences.[1] Investigation on the constituents of the wood was initiated by Kafuku and Ichikawa in 1935,[2] who reported the presence of substance(s) which on saponification afforded benzylacetone and dihydrocinnamic acid. Later, Indian chemists investigated the constituents of the steam distilled oil(agar oil), and disclosed the structures of several sesquiterpenes such as agarospirol,[3a] agarol,[3b] and agarofurans.[3c] In this communication, we describe the isolation and the structure determination of the benzylacetone producing substance which was given the name "agarotetrol".

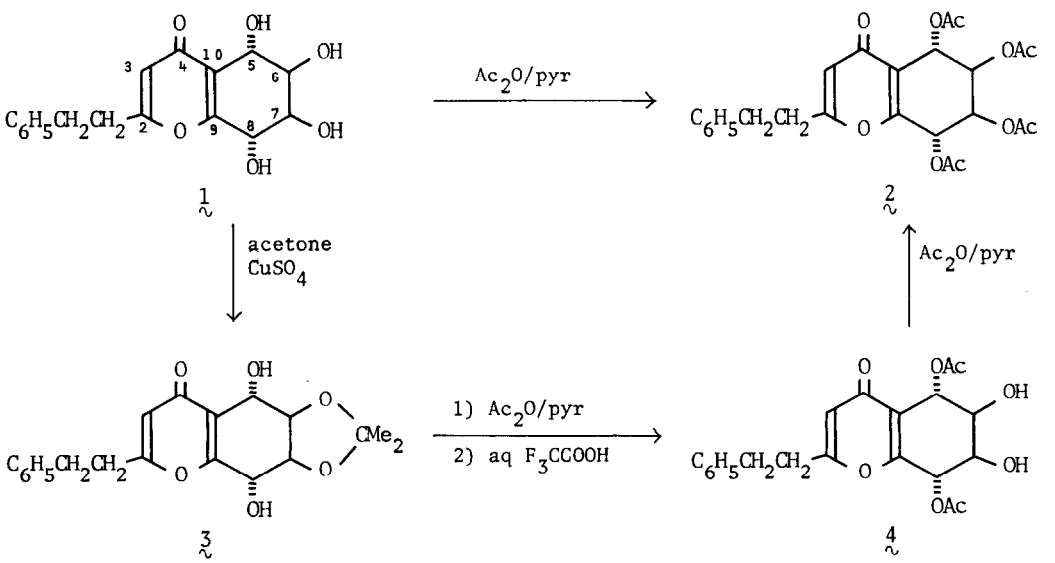
Agarotetrol(1), $C_{17}H_{18}O_6$, mp 118-121°C, $[\alpha]_D$-21.9°(*c* 1.04, MeOH), was isolated from an acetone extract of powdered agarwood by dry column chromatography followed by preparative TLC.[4,5] The spectral data clearly demonstrated the presence of a γ-pyrone ring[IR(KBr) 1660, 1603 cm^{-1}; UV (EtOH) 252 nm(ε 10660)][6] which is 2,3,5-trisubstituted[NMR δ_{ppm} 6.06(s, 1 H),[7] 114.0(d)[8]] and a phenethyl group(NMR, Table 1). The phenethyl group should be located at C_2 of the γ-pyrone ring from the mechanistic consideration of the degradation reaction(*vide supra*). The remaining moiety was proved to consist of four adjacent secondary alcohols by NMR(Table 1) and by the formation of tetraacetate(2).[9] Thus, the planar structure of agarotetrol was revealed to be 2-(2-phenylethyl)-5,6,7,8-tetrahydroxy-5,6,7,8-tetrahydrochromone.

The relative stereochemistry of the four hydroxy groups was then determined as follows. Treatment of 1 with refluxing acetone in the presence of anhydrous $CuSO_4$ afforded a single monoacetonide 3,[10-12] indicating that agarotetrol(1) possesses only one cis glycol system. The base peak *m/e*258 observed in MS of 3 was most reasonably interpreted as the one arising from a retro Diels-Alder fragmentation(M^+–2,2-dimethyl-1,3-dioxole),[13] suggesting the cis glycol be located at C_6 and C_7. This prediction was confirmed by the derivation of the 5,8-diacetate 4[10,14] from 3 by acetylation[10,15] followed by hydrolysis of the acetonide group. Two doublets at δ 5.92 and 6.03_{ppm} observed in 4 were assigned to the acetoxy bearing methine protons, C_5-H and C_8-H respectively, based on the chemical shifts of methine protons of the tetraacetate 2 which were determined by their multiplicity and Eu(dpm)$_3$ induced shifts(Table 1). Thus the relative stereochemistry of the hydroxy groups in 1 should be 5/6 trans, 6/7 cis, and 7/8 trans. The vicinal coupling constants in 2 and 4 were also in accord with this conclusion, assuming a half-chair conformation of the cyclohexene ring[16] in which 5-OAc takes pseudo-axial position as indicated in the structure in Fig. 1.[17]

Table 1. NMR Spectra of Agarotetrol, and Tetra- and Diacetates[a]

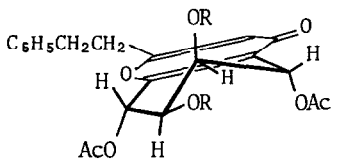
Carbon	Agarotetrol (1)[b]		Tetraacetate (2)		Diacetate (4)
	^{1}H NMR (CD_3OD+C_6D_6=1:3)	^{13}C NMR (CD_3OD)	^{1}H NMR (CCl_4)	ΔEu[c]	^{1}H NMR ($CDCl_3$)[d]
2		171.1(s)			
3	6.06(s)	114.0(d)	6.06(s)	4.4	6.14(s)
4		181.8(s)			
5	4.78(d,J=7.5)	66.8(d)	5.78(d,J=4.5)	9.8	5.92(d,J=3.7)
6	4.32(diff s)	70.1(d)	5.38(dd,J=4.5,3.0)	4.0	4.10(dd,J=3.7,2.7)
7	4.26(diff s)	72.5(d)	5.31(dd,J=8.2,3.0)	3.8	4.04(dd,J=8.2,2.7)
8	5.05(d,J=4.2)	73.9(d)	5.90(d,J=8.2)	2.4	6.03(d,J=8.2)
9		165.1(s)			
10		141.1(s)			
$-CH_2CH_2-$	2.72(m)	36.2(t),33.7(t)	2.87(m)	0.5	2.90(m)
$-C_6H_5$	7.18(m)	121.7(s),129.5(d)	7.22(m)		7.27(m)
		129.3(d),127.3(d)			
$-COCH_3$			2.17(s),2.13(s)		2.07(s),2.23(s)
			2.08(s),2.06(s)		

a Multiplicity: s,singlet; d,doublet; dd,doublet of doublets; t,triplet; q,quartet; m,multiplet; diff,diffused. Multiplicity in carbon NMR obtained through NOE mode. Chemical shifts are in units relative to tetramethylsilane. Coupling constants(J) are expressed in Hz. *b* Assignments of C_5-H to C_8-H may be interchangeable. The preferred conformation of 1 seems to be different from those of 2 and 4, presumably owing to intramolecular hydrogen bondings. *c* P.V. Demarco, T.K. Elzey, R.B. Lewis, and E. Wenkert, *J.Am.Chem.Soc.*, 92, 5734, 5737(1970). *d* CD_3OD was added.



The absolute configuration of agarotetrol(1) was determined by the exciton chirality method.[18] The di-*p*-methoxybenzoate(6)[19] obtained by *p*-methoxybenzoylation of 4 was shown by NMR[19] to take the same preferred conformation as 2 and 4. The split Cotton effects shown in Fig. 2 indicated a negative chirality of the dibenzoate groups giving the absolute configuration depicted in the structure 1.

It should be noted that agarotetrol has an unprecedent novel structure in that it bears hitherto unknown 2-phenethylchromone system and also in its high oxidation level.



2: R = Ac
4: R = H
6: R = *p*-$MeOC_6H_4CO$

Fig. 1. Conformation of Agarotetrol Derivatives

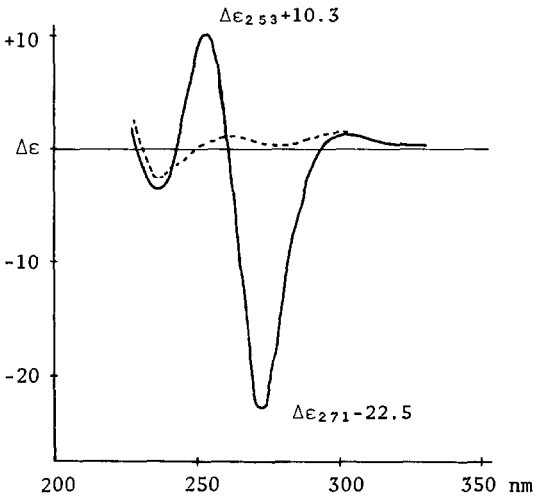


Fig. 2. CD Spectra(MeOH)
4: ---------
6: ————

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References and Notes

1) Sadgopal and B.S. Varma, *Soap, Perf. & Cosmetics*, 25, 169(1952).
2) K. Kafuku and N. Ichikawa, *Nippon Kagaku Zasshi*, 56, 1155(1935); N. Ichikawa and H. Yo, *Ibid.*, 60, 1247(1939).
3) (a) K.R. Varma, M.L. Maheshwari, and S.C. Bhattacharyya, *Tetrahedron*, 21, 115(1965). (b) T.C. Jain and S.C. Bhattacharyya, *Tetrahedron Lett.*, 13(1959); K.R. Varma and S.C. Bhattacharyya, *Tetrahedron*, 20, 2927(1964). (c) M.L. Maheshwari, T.C. Jain, R.B. Bates, and S.C. Bhattacharyya, *Tetrahedron*, 19, 1079(1963); M.L. Maheshwari, K.R. Varma, and S.C. Bhattacharyya, *Ibid.*, 19, 1519(1963).
4) B. Loev and M.M. Goodman, *Chem. Ind.*, 2026(1967).
5) Powdered agarwood was extracted with acetone five times at room temperature. The dark brown viscous oil obtained after evaporation of the solvent was deposited on silica gel of about two fold weight. The mixture was charged on a column(3×65 cm, Nylon) of silica gel(Merck, about eight times weight of the mixture) and the column was eluted with dichloromethane. A top zone was extracted and the extract was subjected to the same chromatography again but

using dichloromethane/MeOH=9 as eluent. The components constituting the band down to *Rf*=0.25 was recovered by extraction with MeOH-dichloromethane and then subjected to preparative TLC (Merck silica gel $PF_{254+366}$, chloroform/MeOH=5) twice. Agarotetrol was obtained from the band *Rf*=ca. 0.4(254 nm detection) as an amorphous solid and yielded benzylacetone on heating with 5% methanolic NaOH. Agarotetrol was hardly crystallizable giving gelatinous colonies from chloroform-MeOH which on drying in vacuo gave a powder. The homogeneity of the sample was confirmed by TLC, HPLC(Toyo Soda ODS column, 0.4×30 cm, 70% MeOH, *Rt*=4.0 min) and GLC of the trimethylsilyl ether(OV-17 1% on Gaschrom Q 80/100 mesh, 4 mm×1.5 m, 220°C, FID detection, relative *Rt*=0.32 to cholesterol acetate=32 min). MS(high resolution) *m/e* 319.1185(calcd for M^++1=319.1182); MS(low resolution) *m/e*(rel abundance) 318(M^+, 4), 300(12), 282(100), 258(68), 91(81).

Quantitative determination of agarotetrol by GLC of the trimethylsilyl ether(internal standard, cholesterol acetate) indicated that agarwood contained 0.51-3.67% agarotetrol depending on the market where purchased.

6) K. Yamada, *Bull. Chem. Soc. Japan*, 35, 1323(1962).
7) L.C. Dorman, *J. Org. Chem.*, 32, 4105(1967); D.W. Mayo, P.J. Sapienza, R.C. Lord, and W.D. Phillips, *Ibid.*, 29, 2682(1964).
8) M.J. Loots, L.R. Weingarten, and R.H. Levin, *J. Am. Chem. Soc.*, 98, 4571(1976); C.A. Kingsbury, M. Cliffton, and J.H. Looker, *J. Org. Chem.*, 41, 2777(1976).
9) Tetraacetate(2) obtained by acetylation of 1 or diacetate 4 with acetic anhydride and pyridine was not crystallizable. MS(high resolution) *m/e* 487.1604(calcd for M^++1=487.1604); MS(low resolution)*m/e*(rel abundance) 487(M^++1, 3) 486(M^+, 5), 443(70), 426(12), 384(76), 383(38), 341 (50), 325(80), 324(64), 282(100), 91(98); IR(neat) 1755, 1670, 1640 cm^{-1}; UV(EtOH) 250 nm(ε 6240); ^{13}C NMR($CDCl_3$) 20.6(CH_3CO), 32.5, 35.1(CH_2CH_2), 63.7, 66.4, 68.2, 69.0($C_{5,6,7,8}$), 114.0 (C_3), 119.2, 126.7, 128.1, 128.7(phenyl), 139.1(C_{10}), 158.8, 168.1($C_{2,9}$), 169.0, 168.9(CH_3CO), 176.5(C_4).
10) All new crystalline compounds gave satisfactory elemental analyses.
11) When toluene-*p*-sulfonic acid was used as a catalyst, a phenolic dehydration product, mp 197.5 -202.5°C, was obtained as a byproduct, to which the structure of 5,8-dihydroxy-2-phenylethyl-chromone was assigned by spectral data: MS(high resolution) *m/e* 282.0923(calcd for $C_{17}H_{14}O_4$= 282.0893); MS(low resolution) *m/e*(rel abundance) 282(M^+, 36), 91(100); IR(KBr) 1655, 1620, 1590, 1570 cm^{-1}; UV(EtOH) nm(ε) 241(12700), 254(sh, 10500), 305(940), 360(2190); ^1H NMR($CDCl_3$) 3.03 (m, CH_2CH_2), 6.09(s, 3-H), 6.60, 7.14(d, *J*=9 Hz, 6- and 7-H), 7.30(m, phenyl).
12) Monoacetonide(3), mp 120-121°C, needles from ether: MS(high resolution) *m/e* 358.1382(calcd for $C_{20}H_{22}O_6$=358.1415); MS(low resolution) *m/e*(rel abundance) 358(M^+, 2), 343(9), 301(3), 300(3), 282(82), 258(100), 253(32), 91(67); IR(KBr) 1660, 1605 cm^{-1}; UV(EtOH) 252 nm(ε 8390); ^1H NMR (CD_3SOCD_3) 1.12, 1.27(gem CH_3), 2.93(diff s, CH_2CH_2), 4.33(diff d, *J*=7.8, 5- or 8-H), 4.60(br s, 6- and 7-H), 4.82(diff d, *J*=6.5, 5- or 8-H), 5.48(d, *J*=6.5, OH), 5.62(d, *J*=7.8, OH), 6.12 (s, 3-H), 7.26(s, phenyl); ^{13}C NMR($CDCl_3$) 24.1, 26.3(gem CH_3), 32.8, 35.4(CH_2CH_2), 62.2, 68.6 ($C_{6,7}$), 77.0, 78.4($C_{5,8}$), 108.6(CH_3-*C*-CH_3), 114.2(C_3), 122.8, 126.6, 128.2, 128.6(phenyl), 139.3(C_{10}), 166.9, 168.7($C_{2,9}$), 178.3(C_4).
13) S.K. Yang, D.W. McCourt, H.V. Gelboin, J.R. Miller, and P.P. Roller, *J. Am. Chem. Soc.*, 99, 5124(1977).
14) Diacetate(4), mp 171.5-173°C. needles from acetone-*iso*Pr_2O: UV(MeOH) nm(ε) 213(14950), 250 (13240); CD(MeOH) Δε(nm) +1.2(299), +0.4(261), -2.8(234).
15) Diacetate of 3, mp 162-163°C, leaflets from acetone-*iso*Pr_2O: IR(KBr) 1755, 1740, 1670, 1630 cm^{-1}; ^1H NMR(C_6D_6) 1.12, 1.20(s, gem CH_3), 1.70, 1.73(s, OAc), 2.05-2.63(m, CH_2CH_2), 4.36 (dd, *J*=2.3, 6.8 Hz, 6-H), 4.57(dd, *J*=1.5, 6.8 Hz, 7-H), 5.87(d, *J*=2.3 Hz, 5-H), 5.90(s, 3-H), 6.68(d, *J*=1.5 Hz, 8-H), 6.80-7.25(m, phenyl).
16) H. Yagi, D.R. Thakker, O. Hernandez, M. Koreeda, and D.M. Jerina, *J. Am. Chem. Soc.*, 99, 1604 (1977).
17) This conformation(Fig. 1) is expected to be more stable than the conformational isomer with 5-H*ax*, since steric and electrostatic repulsions between pyrone carbonyl and 5-OAc are minimized.
18) N. Harada and K. Nakanishi, *Accounts Chem. Res.*, 5, 257(1972).
19) Di-*p*-methoxybenzoate(6), colorless glass: UV(MeOH) nm(ε) 210.5(46470), 258(43530); ^1H NMR (CD_3OD) 2.15, 2.20(s, OAc), 3.00(br s, CH_2CH_2), 3.85, 3.87(OMe), 5.77(dd, *J*=8.1, 2.4 Hz, 7-H), 5.82(dd, *J*=2.4, 3.6 Hz, 6-H), 6.15(d, *J*=3.6 Hz, 5-H), 6.23(s, 3-H), 6.30(d, *J*=8.1 Hz, 8-H), 6.93, 7.00, 7.88, 7.92(d, *J*=9 Hz, *p*-methoxybenzoyl), 7.27(br s, phenyl).

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