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SODIUM BOROHYDRIDE REDUCTION OF ANTHOCYANIDINS*

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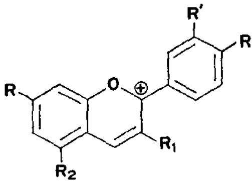
Key Word Index—Anthocyanidins; $NaBH_4$ reduction; 3′,5′-di-*O*-methylepigallocatechin; 3′-*O*-methylepigallocatechin; 3′-*O*-methylepicatechin.

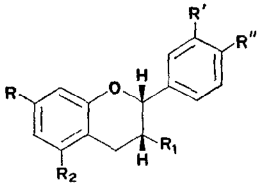
Abstract—The reduction of anthocyanidins with $NaBH_4$ in EtOH or MeOH produces *inter alia* racemates of epicatechins. Thus, the (±) racemates of 3′,5′-di-*O*-methylepigallocatechin, 3′-*O*-methylepigallocatechin, and 3′-*O*-methylepicatechin have been identified as the reduction products of malvidin, petunidin, and peonidin, respectively, by their UV, MS and NMR spectra.

INTRODUCTION

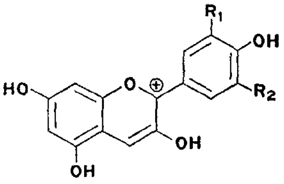
FLAVYLIUM salts (I) in EtOH and MeOH are easily reduced to flavans (II) when treated with PtO_2 in a hydrogen atmosphere.[1] The reduction products isolated after complete hydrogenation of cyanidin (Ia), its pentamethylether (Ib), fisetinidin[2] (Ic) and butinidin[3] (Id) are (±)-epicatechin (IIa), its pentamethyl ether (IIb), (±) 2,3-*cis*-3,3′,4′,7-tetrahydrohydroxyflavan (IIc) and (±) 2,3-*cis*-3′4′,7,-trihydroxyflavan (IId) respectively.



(Ia) R = R$_1$ = R$_2$ = R′ = R″ = OH
(Ib) R = R$_1$ = R$_2$ = R′ = R″ = OMe
(Ic) R = R$_1$ = R′ = R″ = OH ; R$_2$ = H
(Id) R = R′ = R″ = OH ; R$_1$ = R$_2$ = H



(IIa) R = R$_1$ = R$_2$ = R″ = OH
(IIb) R = R$_1$ = R$_2$ = R′ = R″ = OMe
(IIc) R = R$_1$ = R′ = R″ = OH ; R$_2$ = H
(IId) R = R′ = R″ = OH ; R$_1$ = R$_2$ = H



(IIIa) R$_1$ R$_2$ = OMe
(IIIb) R$_1$ = OMe ; R$_2$ = OH
(IIIc) R$_1$ = OMe ; R$_2$ = H

In the process of the reaction, the consumption of the first mol of hydrogen is almost immediate, while that of the second mol requires 30 min[1] showing that the reduction of flavylium salts to flavans is a two-step process. Milder reduction of an anthocyanidin and synthetic flavylium salts with $LiAlH_4$[4] and reduction of synthetic flavylium salts with $NaBH_4$[5] produced flavenes, which are rapidly oxidized in nonaqueous media to flavylium

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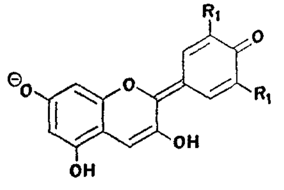
* Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 1924.

[1] K. FREUDENBERG, H. FIKENTSCHER, M. HARDER and O. SCHMIDT, *Liebigs Ann. Chem.* **444**, 135 (1925).
[2] F. E. KING, J. W. CLARK-LEWIS and W. F. FORBES, *J. Chem. Soc.* 2948 (1955).
[3] K. FREUDENBERG and P. MAITLAND, *Liebigs Ann. Chem.* **510**, 193 (1934).
[4] P. KARRER and M. SEYHAN, *Helv. Chim. Acta* **33**, 2209 (1950).
[5] L. JURD, *Tetrahedron* **23**, 1057 (1967).

salts,[5] similar to the product formed by PtO_2 reduction of cyanidin when the reaction was interrupted after the consumption of 1 mol of hydrogen.[1] Catechins or flavans have not heretofore been reported by the reduction of flavylium salts with $LiAlH_4$ or $NaBH_4$.

RESULTS AND DISCUSSION

The alcoholic solutions of the investigated anthocyanidins, i.e. malvidin (IIIa), petunidin (IIIb), and peonidin (IIIc), turned blue immediately upon addition of $NaBH_4$, caused by structural transformation of the pigments as effected by pH[6] to the ionized anhydrobase form IV. The blue color faded rapidly, and after 10 min, the solution appeared colorless.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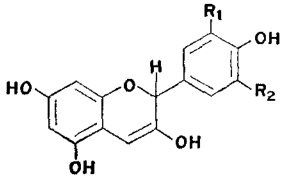
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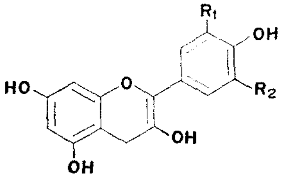
(VI)

TLC of the reaction mixtures of all three pigments showed four major and four minor mobile compounds present, plus an intense purple-colored spot (with Gibbs reagent) which remained at the origin, possibly consisting of oligo- and poly-meric flavonoid.[7]

TABLE 1. $NaBH_4$ REDUCTION PRODUCTS OF ANTHOCYANIDINS

Anthocyanidin	Reduction product		m.p.	λ_{max} (nm) (MeOH)	ϵ	λ_{max} (nm) (MeONa)	ϵ	$[\alpha]_D^{24°}$ (EtOH, $C=1$)
Malvidin	3',5'-di-*O*-methyl-epigallocatechin	$C_{17}H_{18}O_7$	218–221°	271·5	2100	316	2600	−1·0°
				279 sh		515	450	
	-tetraacetate	$C_{25}H_{26}O_{11}$	95–6°	271·5	3250			
				274·4 inf	3125			
				277	3250			
Petunidin	3'-*O*-Methyl-gallocatechin	$C_{16}H_{16}O_7$	138–140°	271	1579	280	2939	+0·20°
				279 sh	1140	316 sh	1620	
	-pentaacetate	$C_{26}H_{26}O_{12}$	94–95°	271	3723			
				276	3829			
Peonidin	3'-*O*-Methyl-epicatechin	$C_{16}H_{16}O_6$	221–223°	279·5	3711	280	4044	+1·4°
						284 sh		
						309 sh		
						337	387	
	-tetraacetate	$C_{24}H_{24}O_{10}$	82–83°	273·5	3548			
				277·5	3490			

The isolated reduction products, (R_f in MeOH–benzene (1:2) 0·44, 0·28 and 0·38, from IIIa, IIIb and IIIc, respectively) crystallized as yellowish prisms (compound from IIIa) or colorless needles (compounds from IIIb and IIIc), which melted at 218–221°, 138–140° and 221–223°, respectively, to deep red-colored liquids. Based on the mild reducing properties

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[6] L. Jurd and T. A. Geissmann, *J. Org. Chem.* **28**, 2394 (1963).

[7] L. Jurd and A. C. Waiss, Jr., *Tetrahedron* **24**, 2801 (1968).

of $NaBH_4$, previous reports in the literature about reduction of flavylium salts with mild reducing agents,[4,5] and the properties of the isolated compounds described above, it was expected that the products formed would be flavenes of type V or VI. TLC of the melted compounds indicated neither the presence of anthocyanidins nor of the original reduction products. In fact, the crystalline reduction products were stable on exposure to air, and in aqueous acid solutions formed red–brown phlobaphenes when allowed to stand at room temperatures. Oxidation of the compounds with bromine,[8] or of their acetyl derivatives with *N*-bromosuccinimide,[9] both reactions which are known to produce anthocyanidins from catechins, produced the original anthocyanidins IIIa, IIIb and IIIc. The spectral properties of the reduction products, and those of their acetyl derivatives (e.g. λ_{max}; ϵ; Table 1), suggested further the flavan character of the isolated compounds.

TABLE 2. MS DATA OF 3′,5′-di-*O*-METHYLEPIGALLOCATECHIN (IXa)

m/e	% Abundance	*m/e*	% Abundance	*m/e*	% Abundance
50	3·0	81	3·4	139	27·8 (VII)
51	6·8	82	3·4	140	3·1
52	2·8	83	3·4	141	2·8
53	6·2	93	3·4	149	3·4
54	3·4	94	5·6	151	4·0
55	13·4	95	3·4	152	2·0
56	5·6	104	2·2	153	6·4
57	6·8	105	2·8	163	3·4
63	3·1	106	2·2	167	42·0
64	3·0	107	3·5	168	20·0
65	6·2	108	1·7	169	3·4
66	3·4	109	2·2	181	6·2
67	4·5	110	3·4	186	6·8
68	2·8	111	2·2	195	5·6
69	9·6	112	2·6	196	100·0 (VIIIa)
70	6·8	115	2·5	197	13·6
71	2·8	121	3·4	315	3·4
77	6·2	122	3·4	317	1·8
78	3·4	123	4·7	334 M+	35·0
79	3·4	137	2·8	335 M+1	6·8
80	1·4	138	2·8	336 M+2	1·7

The MS analysis of the reduction products (Tables 2–4) established their MW as 334, 320 and 304 (from IIIa, IIIb and IIIc respectively), in accord with the elemental composition, and showed the characteristic retrodiene-type fragmentation of flavans.[10] Thus, the $NaBH_4$-reduction product of IIIa produced the trihydroxybenzyl (*m/e* 139, VII) and syringyl-C_2 (*m/e* 196, VIIIa) fragments. Similarly, the MS of the reduction products from IIIb and IIIc contained VII, the monomethoxygallyl-C_2 (*m/e* 182, VIIIb) and guaiacyl-C_2 (*m/e* 166, VIIIc) fragments respectively.

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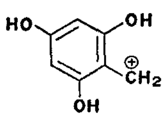
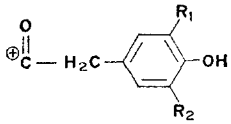
[8] H. APPEL and R. ROBINSON, *J. Chem. Soc.* 426 (1935).

[9] A. K. GANGULI, T. R. SESHADRI and P. SUBRAMANIAN, *Proc. Indian Acad. Sci.* **46A**, 25 (1957).

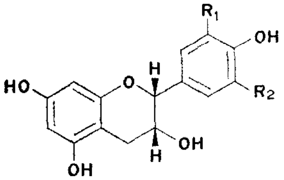
[10] K. WEINGES, W. BAEHR, W. EBERT, K. GOERITZ and H. D. MARX, in *Progress in the Chemistry of Organic Natural Products* (edited by L. ZECHMEISTER), Vol. 27, p. 198, Springer, Berlin (1969).



(VII)



(VIIIa) $R_1 = R_2 = OMe$
(VIIIb) $R_1 = OMe$; $R_2 = OH$
(VIIIc) $R_1 = OMe$; $R_2 = H$



(IXa) $R_1 = R_2 = OMe$
(IXb) $R_1 = OMe$; $R_2 = OH$
(IXc) $R_1 = OMe$; $R_2 = H$

The protons on the heterocyclic ring of all three compounds were similar and almost identical with those of (—)-epicatechin pentaacetate and showed considerable differences of those from (+)-catechin pentaacetate. Thus, the C-2 protons gave rise to a broad singlet, due to weak coupling with the aromatic protons of the 2-aryl group,[11] and were well resolved and separated from the C-3 protons (differences in chemical shifts larger than 30 Hz). The two protons of the 4-methylene group absorbed as a closely spaced doublet near 2·9 ppm, in agreement with a 2,3-*cis* configuration.[11,12]

TABLE 3. MS DATA OF 3′-*O*-METHYLEPIGALLOCATECHIN (IXb)

m/e	% Abundance	*m/e*	% Abundance	*m/e*	% Abundance
50	5·6	91	2·7	142	4·1
51	9·7	92	4·1	150	2·7
53	5·5	93	5·5	151	6·9
54	5·5	94	11·1	152	5·5
55	18·0	95	4·8	153	44·4
56	6·9	96	2·7	154	25·0
57	11·1	97	5·5	155	6·9
63	5·6	104	2·8	165	2·7
64	7·6	105	3·5	166	16·6
65	11·1	106	2·1	167	13·9
66	5·5	107	2·7	168	16·6
67	5·6	108	2·1	169	4·1
68	4·1	109	4·1	179	2·7
69	18·1	110	8·3	180	5·5
70	5·5	111	6·9	181	8·3
71	6·9	115	5·5	182	93·0 (VIIIb)
77	9·7	121	4·1	183	9·7
78	6·2	122	4·1	184	2·7
79	4·1	123	5·5	185	2·7
81	6·9	137	9·0	302	4·1
82	4·1	138	13·9	304	5·5
83	4·1	139	100·0 (VII)	320 M+	36·1
84	4·1	140	11·1	321 M+1	5·6
85	4·1	141	9·7	322 M+2	1·1

Since all three compounds showed very little optical activity (Table 1), and the NMR data obtained prove (Table 5) that the flavans are of the *epi*-series, their presence as the (±)-racemates can be safely assumed. Following the proposed nomenclature of Freudenberg and Weinges[13] the names of 3′,5′-di-*O*-methylepidelphidanol, 3′-*O*-methylepidelphidanol

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[11] J. B. CLARK-LEWIS, L. M. JACKMAN and T. M. SPOTSWOOD, *Austral. J. Chem.* **17**, 632 (1964).
[12] J. B. CLARK-LEWIS, *Austral. J. Chem.* **21**, 2059 (1968).
[13] K. FREUDENBERG and K. WEINGES, *Tetrahedron* **8**, 336 (1960).

and 3'-*O*-methylepicyanidanol are suggested for 3',5'-*O*-methylepigallocatechin (IXa), 3'-*O*-methylepigallocatechin (IXb) and 3'-*O*-methylepicatechin (IXc), respectively.

TABLE 4. MS DATA OF 3'-*O*-METHYLEPICATECHIN (IXc)

m/e	% Abundance	*m/e*	% Abundance	*m/e*	% Abundance
52	7·6	95	7·6	153	1·9
53	3·8	121	2·5	163	1·2
54	1·2	122	5·1	164	1·9
55	3·8	123	7·6	165	2·5
63	1·2	137	50·0	166	100·0 (VIIIc)
65	7·6	138	24·3	167	16·6
66	2·5	139	82·0 (VII]	168	1·2
67	3·8	140	7·0	178	2·5
69	10·2	141	7·0	274	2·5
77	5·1	142	1·2	285	2·5
78	1·2	143	2·5	286	5·1
79	1·2	149	2·5	304 M+	33·3
81	2·5	150	3·8	305 M+1	6·4
93	2·5	151	10·2	306 M+2	1·2
94	2·5	152	2·5		

It is well known that mild reduction of synthetic flavylium salts containing fewer hydroxyl groups in the molecule than naturally occurring anthocyanidins yield flavenes.[4,5] The production of flavanols from the anthocyanidins studied can possibly be explained by reduction of the latter to flavenes, which are in equilibrium with the 3-keto form and which undergo a further reduction of the 3-carbonyl to yield flavans.[14] If the actual reaction follows this path, the reduction of the 3-carbonyl group of the flavene equilibrium product has to be instantaneous, since TLC investigation of the reaction mixture showed the presence of flavans immediately after the addition of $NaBH_4$, together with 3 other compounds present in approximately equal amounts. No information could be obtained about reaction products other than the reported flavans because of difficulties encountered in purification and isolation. Their extreme lability suggest that they may be flavenes of type V and VI and possibly their hydrolysis products.

TABLE 5. CHEMICAL SHIFTS OF A, B AND C-RING PROTONS OF THE CATECHIN DERIVATIVES

						δ (ppm)				
Compound	3-OAc	2H	3H	4H 4*H	6H	8H	2'H 6'H	5'H	Aromatic-OAc	3'OMe 5'-OMe
(+)-3,5,7,4'-Acetoxy-3,5-di-*O*-methylepigallocatechin§	1·86	5·31	4·85	2·78†	6·45‡	6·61‡	6·54		2·24	3·74
(+)-3,5,7,4',5'-Acetoxy-3'-*O*-methylepigallocatechin	1·92	5·40	5·06	2·92†	6·55‡	6·67‡	6·90		2·28–2·30	3·84
(+)-3,5,7,4'-Acetoxy-3-*O*-methylepicatechin	1·90	5·38	5·07	2·92†	6·53‡	6·67‡	6·98	7·06	2·26–2·30	3·83
(−)-3,5,7,3',4'-Acetoxy-epicatechin	1·89	5·39	5·08	2·89†	6·53‡	6·64‡	7·19	7·29	2·27	—
(+)-3,5,7,3',4'-Acetoxy-catechin§	1·95		5·10*	2·75†	6·52‡	6·59‡	7·18	7·10	2·23	—

* Centre of multiplet. † Centre of doublet. ‡ $J = 2$ Hz. 4* = 4-quasi axial. § In CCl_4, remainder in $CDCl_3$.

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[14] L. Jurd, private communication.

EXPERIMENTAL

Isolation of anthocyanins. Malvidin-and petunidin-3,5-*diglucosides.* Freshly prepared juice (18 l.) of Seibel 9549 grapes, harvested immediately before processing, was filtered and percolated through a Sephadex K 100 column, packed with polyvinylpyrrolidone powder in H_2O. The pigments were separated, isolated, and crystallized as reported in a previous paper.[15] Yield: 10·40 g malvidin-3,5-diglucoside, 5·04 g petunidin-3,5-diglucoside.

Peonidin-3,5-*diglucoside.* Fresh petal leafs (2800 g) of *Paeonia officinalis* var. Richard Carwell were extracted in the cold with 2% methanolic HCl (19 l.) for 16 hr, and filtered. The solution was concentrated to *ca.* 4000 ml and filtered again. Et_2O was added to the filtrate until precipitation occurred, the solution was stored overnight at 4°, and the precipitated pigment was collected. Crystallized from 0·5 N HCl as fine, red–violet needles. Yield: 6·90 g.

Hydrolysis of the anthocyanins. The 3,5-diglucosides were hydrolysed with either 3 N HCl for 45 min or 6 N HCl for 30 min. The anthocyanidins so obtained were recrystallized from aq. HCl and shown to be chromatographically pure.

3′,5′-*di*-O-*methylepigallocatechin* (IXa). $NaBH_4$ (0·5 g) was added in portions during 10 min to a cooled solution of malvidin (1·0 g) in EtOH (10 ml) and 1% $NaHSO_3$ aq. (100 ml) was added. The EtOH was evaporated; the aqueous solution was extracted with EtOAc (4 × 50 ml), the EtOAc extract was dried and evaporated to dryness. The residue was dissolved in a 10-ml MeOH–benzene (1:2) and chromatographed on a silica gel column in MeOH–benzene. TLC of the fractions in MeOH–benzene (1:2) showed that the first major compound (IXa) eluted from the column (R_f 0·44, purple with Gibbs) contained impurities. Rechromatography of fractions containing VIIa on a silica gel column with EtOH–benzene (1:2) gave the pure compound. Crystallized from aq. EtOH and recrystallized from H_2O as yellowish prisms, yield: 100 mg, m.p. 218–221°. The compound decomposed rapidly in MeONa and its alkali spectrum with H_3BO_3 + NaOAc was the same as in MeOH. M^+: 334 (Found: C, 61·18; H, 5·47. Calc. for $C_{17}H_{18}O_7$: C, 61·13; H, 5·43). IXa formed a tetraacetate, which crystallized from MeOH–H_2O as microcrystalline prisms, m.p. 95–96° (Found: C, 59·89, H, 5·31. Calc. for $C_{25}H_{26}O_{11}$: C, 59·75; H, 5·21%). R_f: 0·77 (silica gel, MeOH–benzene (1:2). Oxidation of IXa in dioxan with bromine[8] and that of tetraacetyl–IXa in CCl_4 with *N*-bromosuccinimide[9] provided malvidin, identical with the authentic pigment on cellulose TLC in 3 solvents.

3′-*O-methylepigallocatechin* (IXb). $NaBH_4$ (460 mg) was added in portions during 10 min to a cooled solution of petunidin (926 mg) in EtOH (10 ml) and processed as above. After chromatography and rechromatography on silica gel with MeOH–benzene (1:2), pure IXb was obtained, which crystallized from H_2O as fine needles. Yield: 100·4 mg, m.p. 138–140°, R_f 0·28 (in MeOH–benzene (1:2); rapidly decomposing in NaOMe solution and turning yellow. M^+: 320 (Found: C, 60·11, H, 5·08 Calc. for $C_{16}H_{16}O_7$: C, 60·05; H, 5·04%). IXb formed the pentaacetate, obtained as a microcrystalline powder from MeOH–H_2O, m.p. 94–95° (Found: C, 58·89; H, 4·86°. Calc. for $C_{26}H_{26}O_{12}$: C, 58·91; H, 4·94%). R_f 0·43 (in MeOH–, benzene, 1:2). Oxidation of IXb in dioxan with bromine and of the pentaacetyl–IXb in CCl_4 with *N*-bromosuccinimide provided petunidin, identified on cellulose TLC in 3 solvents with authentic material.

3′-O-*methylepicatechin* (IXc). $NaBH_4$ (360 mg) was added in portions during 10 min to a cooled solution of peonidin (720 mg) in EtOH (10 ml), and the reaction-mixture processed as above. After repeated chromatography on silica gel with MeOH–benzene (1:2), Et_2O, and EtOH–benzene (1:2), pure IXC was obtained. Crystallized from H_2O in colorless prisms; Yield: 34 mg, m.p. 221–223°, R_f 0·39 (on silica gel, MeOH–benzene 1:2) and 0·14 (ethyl ether). M^+: 304 (Found: C, 63·18; H, 5·27. Calc. for $C_{16}H_{16}O_6$: C, 63·21; H, 5·30%). IXc formed the tetraacetate which was obtained as a microcrystalline powder from MeOH–H_2O, m.p. 82–83° (Found: C, 60·85; H, 5·18. Calc. for $C_{24}H_{24}O_{10}$: C, 60·93; H, 5·12%). R_f 0·57 (in MeOH–benzene, 1:2). Oxidation of IXc in dioxan with bromine and of the tetraacetyl IXc in CCl_4 with *N*-bromosuccinimide provided peonidin identical on cellulose TLC with the authentic pigment in the solvent systems used above. (+)-Catechin (100 mg) and (−)-epicatechin (100 mg) were acetylated in pyridine with AcOAc in the cold and their NMR spectra were recorded for comparison.

Acknowledgements—The author is indebted to Dr. J. C. Moyer for processing of the grapes and grape juice, to Dr. T. E. Acree and Mr. D. C. Angell for recording the NMR and MS and to Miss A. J. Borzell for technical assistance.

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[15] G. HRAZDINA, *J. Agric. Food Chem.* **18**, 243 (1970).