Condensed Tannins

OPTICALLY ACTIVE DIASTEREOMERS OF (+)-MOLLISACACIDIN BY EPIMERIZATION

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(Received 29 July 1964)

1. (+)-Mollisacacidin [(+)-3',4',7-trihydroxy-2,3-*trans*-flavan-3,4-*trans*-diol] is converted by autoclaving into the optically active free phenolic 2,3-*trans*-3,4-*cis* (12% yield), 2,3-*cis*-3,4-*trans* (11%) and 2,3-*cis*-3,4-*cis* (2.8%) diastereoisomers through epimerization at C-2 and C-4. 2. The relative configurations of the epimeric forms were determined by nuclear-magnetic-resonance spectrometry and paper ionophoresis in comparison with synthetic reference compounds, and was confirmed by chemical interconversions. 3. From this a scheme of epimerization is inferred and their absolute configurations are assigned as (2*R*:3*S*:4*S*), (2*S*:3*S*:4*R*) and (2*S*:3*S*:4*S*) respectively from the known absolute configuration (2*R*:3*S*:4*R*) of (+)-mollisacacidin.

(+)-Mollisacacidin [(2*R*:3*S*:4*R*)-3',4',7-trihydroxyflavan-3,4-diol] (I) from the heartwood of *Acacia mearnsii* and related wattles (Keppler, 1957; Roux, Maihs & Paulus, 1961) has a 2,3-*trans*-3,4-*trans* arrangement of substituents in the heterocyclic ring, and the absolute configuration as indicated (Clark-Lewis & Williams, 1963; Drewes & Roux, 1964a).

Comparison of the nuclear-magnetic-resonance spectra of derivatives of this compound with those of its geometrical isomers is useful for further study of dimeric and other polymeric tannins that accompany it in the wood and bark of black wattle (*A. mearnsii*). Thus the trimethyl ethers of isomeric 2,3-*trans*-3,4-*trans*, 2,3-*trans*-3,4-*cis* and 2,3-*cis*-3,4-*cis* racemates were prepared and examined through their oxidation rates with periodate and lead tetra-acetate, and by nuclear-magnetic-resonance spectrometry of their 3,4-diacetates (Clark-Lewis & Williams, 1963; Drewes & Roux, 1964a,b). However, the 2,3-*cis*-3,4-*trans* racemates of highly substituted flavan-3,4-diols are not readily accessible.

Free phenolic or optically pure forms of the diastereoisomers of natural flavan-3,4-diols, which are useful for studying their interconversions and comparing their chemical reactivity, have hitherto not been synthesized. These are now available by epimerization of the optically pure (+)-2,3-*trans*-3,4-*trans* form (Drewes & Roux, 1964d), and the present paper describes details of the stereoisomeric interconversions of (+)-mollisacacidin.

EXPERIMENTAL AND RESULTS

Nuclear-magnetic-resonance spectra were recorded by Dr K. Pachler, Chemical-Physics Group, C.S.I.R., Pretoria, on a Varian A-60 spectrometer with deuteriochloroform as chloroform and tetramethylsilane as internal standard. The sample concentration was 50–100 mg./0.5 ml. of solution. Band positions are expressed as p.p.m. downfield with the standard as origin. Coupling constants were measured with an accuracy of ± 0.2 cye./sec. C, H, methoxyl and acetyl determinations were by K. Jones, Micro-analytical Laboratory, C.S.I.R., Pretoria. All melting points are uncorrected, and determinations of mixed melting points were performed as described by Roux & Maihs (1960).

Two-dimensional chromatograms were run in water-satd. butan-2-ol and then 2% (v/v) acetic acid on Whatman no. 1 paper. Preparative paper chromatograms were on (18½ in. × 22½ in.) sheets of Whatman no. 3 paper. The yields of diastereoisomers, and the recovery of the starting material, are averages for 25 separations.

Isolation of (+)-mollisacacidin

Black-wattle-heartwood drillings (4.5 kg.) were twice extracted in the cold with formaldehyde-free methanol (20l.) over a 7-day period, and the extract was evaporated to dryness under vacuum. The solids (179 g.) were repeatedly extracted with petroleum (b.p. 60–80°) to remove waxes (cf. Roux & Paulus, 1962). The wax-free extract was subjected to countercurrent separation in a Craig machine by using the upper and lower phases of a water–butan-2-ol–petroleum (b.p. 60–80°) (5:4:1, by vol.) mixture. After 160 transfers (+)-mollisacacidin was distributed in tubes 91–120. The solids (31.5 g.) from these fractions were streaked on to Whatman no. 3 sheets (250 mg./sheet) and

the chromatograms developed with 2% (v/v) acetic acid. Bands with R_F 0.60 were located with ammoniacal silver nitrate and toluene-*p*-sulphonic acid spray reagents. These were cut out and eluted with 70% (v/v) ethanol. From the combined eluates (+)-mollisacacidin (7.5 g.), m.p. 127–128°, was obtained.

Method of epimerization

(+)-Mollisacacidin (500 mg.) was dissolved in 50 ml. of hot water and placed in a 6-quart Presto domestic pressure cooker (internal volume approx. 5.5 l.) containing boiling water. The pressure cooker was sealed, and heating was maintained to provide a steam pressure of 15 lb./in.² for 2 hr. The cooker was cooled rapidly under a tap. The almost colourless solution tended to darken rapidly on standing. Two-dimensional chromatograms showed the presence of three main reducing areas when sprayed with ammoniacal silver nitrate, corresponding to R_F values 0.50 and 0.41, 0.60 and 0.51, and 0.71 and 0.60 in water-satd. butan-2-ol and 2% (v/v) acetic acid respectively.

The solution was diluted to 75 ml. with ethanol and streaked on to 15 sheets (5 ml./sheet) of Whatman no. 3 paper, and the chromatograms were developed in 2% (v/v) acetic acid. Three reducing bands having R_F values about 0.40, 0.50 and 0.60, and increasing in intensity in this order, were cut out and eluted with 70% (v/v) ethanol.

(+)-3',4',7-Trihydroxy-2,3-*cis*-flavan-3,4-*cis*-diol

From the band with the lowest R_F (0.40) a brown powder (47 mg.) was obtained. This was dissolved in methanol (15 ml.) and streaked on to three sheets of Whatman no. 3 paper, and the chromatograms were developed with water-satd. butan-2-ol. In most epimerizations the eluates were merely concentrated to small volume and then purified by partition paper chromatography as above. Strongly reducing bands at R_F 0.53 were cut out and eluted with 70% (v/v) ethanol. The combined eluates, when concentrated to small volume under vacuum at 45°, gave fine white needles (14 mg.), m.p. 105–106°, $[\alpha]_D^{20} + 48.5^\circ$ [c 0.8 in acetone–water (1:1, v/v)] (Found: C, 54.9; H, 5.9; loss at 110° for 2 hr. under vacuum, 11.1. $C_{15}H_{14}O_6 \cdot 2H_2O$ requires C, 55.2; H, 5.5; H_2O , 11.0%). The anhydrous compound sintered at 65–67° and melted at 146–150° (decomp.), becoming intensely purple–red at this temperature (Found: C, 63.0; H, 5.3. $C_{15}H_{14}O_6$ requires C, 62.1; H, 4.9%). The compound migrated as a single discrete spot on two-dimensional chromatograms.

(+)-3',4',7-Trimethoxy-2,3-*cis*-flavan-3,4-*cis*-diol. The 2,3-*cis*-3,4-*cis*-leuco-fisetinidin (104 mg.) was methylated with diazomethane at 0° in methanol–ether for 48 hr. Fine rosettes of elongated plates (76 mg.) were obtained from ethanol, m.p. 134°, $[\alpha]_D^{19} + 58.8^\circ$ [c 0.8 in acetone–water (1:1, v/v)] (Found: C, 64.0; H, 6.3; O·CH₃, 30.4. $C_{18}H_{20}O_6$ requires C, 65.1; H, 6.1; O·CH₃, 28.0%).

The corresponding racemate, m.p. 149° (Drewes & Roux, 1964b), when mixed with the above, gave m.p. 132–134°.

(+)-3,4-*cis*-Diacetoxy-3',4',7-trimethoxy-2,3-*cis*-flavan. The trimethyl ether (63 mg.) was acetylated with acetic anhydride–pyridine (1:1, v/v) (0.4 ml.) and crystallized from ethanol, giving white prisms (55 mg.), m.p. 152–153°,

after recrystallization from the same solvent (Found: C, 63.6; H, 5.9; CO·CH₃, 20.2. $C_{22}H_{24}O_8$ requires C, 63.5; H, 5.8; CO·CH₃, 20.7%).

The nuclear-magnetic-resonance spectrum of the diacetate in comparison with synthetic (±)-3,4-*cis*-diacetoxy-3',4',7-trimethoxy-2,3-*cis*-flavan, m.p. 129° (Clark-Lewis & Williams, 1963; Drewes & Roux, 1964b), confirmed its 2,3-*cis*-3,4-*cis* configuration (cf. Table 1). A mixture of these compounds had m.p. 127–129°.

(+)-3'-4',7-Trihydroxy-2,3-*cis*-flavan-3,4-*trans*-diol

The eluates from the band with R_F 0.50 were concentrated to dryness under vacuum at 45° to give a brown powder (93 mg.). The compound was purified by separation on three sheets of Whatman no. 3 paper, with water-satd. butan-2-ol as irrigant. A reducing band, located when sprayed with ammoniacal silver nitrate, at R_F 0.63 was cut out and eluted with ethanol (70%, v/v). Concentration of the eluates, as described above, gave 55 mg. of an amorphous brown powder. Two-dimensional paper chromatography of the product indicated that the diol still contained as impurity a compound having a higher R_F in both directions. The impurity proved to be the 4-ethyl ether. Chromatographic purity of the 2,3-*cis*-3,4-*trans*-diol was achieved by repeating the separation on paper sheets with water-satd. butan-2-ol and eluting with acetone (70%, v/v) instead of ethanol. The amorphous brown powder failed to crystallize; it melted slowly from 65° upwards, decomposing sharply at 115–118°. At this temperature the compound darkened but did not redden intensely as did the 2,3-*cis*-3,4-*cis* and 2,3-*trans*-3,4-*trans* isomers.

No analyses were attempted, owing to difficulty in drying the unstable compound. The compound migrated as a single discrete spot on two-dimensional chromatograms.

(+)-3',4',7-Trimethoxy-2,3-*cis*-flavan-3,4-*trans*-diol. The diol (120 mg.) was methylated in ethereal solution immediately after purification. An amorphous brown solid melting over the range 30–40° was recovered. The product failed to crystallize.

The low melting point precluded satisfactory analyses.

(+)-3,4-*trans*-Diacetoxy-3',4',7-trimethoxy-2,3-*cis*-flavan. The amorphous trimethyl ether (70 mg.) was dried over P₂O₅ and acetylated at room temperature with acetic anhydride–pyridine (1:1, v/v) (0.5 ml.). A white solid (74 mg.) that hardened rapidly was obtained on pouring into water. The compound, which failed to crystallize, sintered at 50° and melted at 75–80° (Found: C, 63.7; H, 6.2; O·CH₃, 22.6. $C_{22}H_{24}O_8$ requires C, 63.5; H, 5.8; O·CH₃, 22.4%).

The nuclear-magnetic-resonance spectrum of the diacetate confirmed its purity, and proved its 2,3-*cis*-3,4-*trans* configuration (cf. Table 1 and the Discussion section).

4-Ethyl ether of (+)-3',4',7-trihydroxy-2,3-*cis*-flavan-3,4-*trans*-diol. The 2,3-*cis*-3,4-*trans*-diol (180 mg.), after one adsorption [2% (v/v) acetic acid] and one partition (water-satd. butan-2-ol) separation, was refluxed for 1.5 hr. in ethanol (5 ml.) containing 1% (v/v) of acetic acid. Two-dimensional chromatograms indicated an approx. 2:3 mixture of the 4-ethyl ether and the free diol. The solution was diluted to 30 ml. with ethanol, streaked on to six pre-washed (with water) cellulose sheets and developed with water-satd. butan-2-ol. The reducing band with the

higher R_F (0.8–0.9) was cut out, eluted and concentrated to small volume at 45°. The solution, when placed under slight vacuum, gave clusters of needles (30 mg.), m.p. 184°. These were recrystallized from ethanol–water (1:1, v/v) to give needles, m.p. 187–188° (decomp.), $[\alpha]_D^{20} + 21.8^\circ$ (c 0.6 in ethanol) (Found: C, 62.6; H, 5.7; O, C₂H₅, 15.3; loss at 140° under vacuum, 3.3. C₁₇H₁₈O₆·½H₂O requires C, 62.4; H, 5.8; O, C₂H₅, 13.8; H₂O, 2.8%). At its melting point the compound becomes dark purple.

(+)-3',4',7-Trihydroxy-2,3-trans-
flavan-3,4-cis-diol

Examination by paper electrophoresis (Drewes & Roux, 1964b) after methylation of the epimerization products of the band with the highest R_F [0.71 in water-satd. butan-2-ol, 0.60 in 2% (v/v) acetic acid] indicated the presence of two diols having 3,4-*cis* and 3,4-*trans* configurations. The eluates from this band, R_F 0.60, were concentrated to small volume at 45°. Fine needles (160 mg.), m.p. 112–126°, separated. From the mother liquor more needles (40 mg.), m.p. 106–110°, were obtained.

Purification of this mixture of two diols, m.p. 106–110°, was effected by streaking 240 mg. in 60 ml. of methanol on to 12 sheets of Whatman no. 3 paper, and developing the chromatogram with phenol–water (3:1, w/v) for 24 hr. Two reducing bands located at R_F 0.47 and 0.53 were cut out and eluted. The eluates from the band with the higher R_F were again streaked on to three cellulose sheets and developed in the same solvent. From this separation a brown powder (40 mg.) was obtained, which was pure by two-dimensional chromatography and by chromatography in phenol–water (3:1, w/v), but which failed to crystallize, m.p. 65° onwards after sintering at 55°. The compound reddened slightly at this temperature, but to a somewhat deeper pink than did the 2,3-*cis*-3,4-*trans* diastereoisomer.

(+)-3',4',7-Trimethoxy-2,3-trans-flavan-3,4-cis-diol. Crystals (225 mg.), m.p. 106–110°, from mother liquors (see above) of the band with the highest R_F [0.60 in 2% (v/v) acetic acid] obtained from the epimerization, were methylated with diazomethane at 0° in a methanol–ether mixture. The solution was concentrated to small volume (2 ml.), when white needles (85 mg.), m.p. 177–180°, separated. These were recrystallized from ethanol, m.p. 186–187°, $[\alpha]_D^{20} + 42.9^\circ$ [c 0.6 in acetone–water (9:5, v/v)] (Found: C, 65.0; H, 6.3; O, CH₃, 29.0. C₁₈H₂₀O₈ requires C, 65.1; H, 6.1; O, CH₃, 28.0%), mixed m.p. 179–180° with the corresponding racemate, m.p. 185° (Drewes & Roux, 1964a).

A few drops of water added to the first mother liquor gave a further crop of crystals (132 mg.), m.p. 129°, after sintering at 94°. This proved to be the trimethyl ether of the starting material (see below).

(+)-3,4-cis-Diacetoxy-3',4',7-trimethoxy-2,3-trans-flavan. The trimethyl ether (70 mg.) was acetylated at room temperature as above, and the mixture poured into water. A white amorphous solid (82 mg.), which sintered at 50° and melted at 80°, was recovered (Found: C, 63.4; H, 5.9; O, CH₃, 22.5; CO, CH₃, 20.7. C₂₂H₂₄O₈ requires C, 63.5; H, 5.8; O, CH₃, 22.4; CO, CH₃, 20.7%).

The nuclear-magnetic-resonance spectrum of this diacetate confirmed its 2,3-*trans*-3,4-*cis* configuration (cf. Table 1).

Recovery of (+)-3',4',7-trihydroxy-2,3-trans-
flavan-3,4-trans-diol

The starting material, (+)-mollisacacidin, was recovered either through repeated recrystallization from water of the crystals, m.p. 112–116°, from the band with the highest R_F [0.60 in 2% (v/v) acetic acid] of the epimerization mixture, or by paper-chromatographic separation of this fraction with phenol–water (3:1, w/v). From 500 mg. of starting material 200 mg. of the mixture of 2,3-*trans*-3,4-*trans*- and 2,3-*trans*-3,4-*cis*-diols was obtained, the former comprising approx. 70% of the mixture. The recovered pure diol, m.p. 125–130°, $[\alpha]_D^{20} + 27.3^\circ$ [c 0.8 in acetone–water (1:1, v/v)] showed no depressing of melting point when mixed with authentic (+)-mollisacacidin. The trimethyl ether of the recovered diol had m.p. 129–130°, $[\alpha]_D^{20} - 9.5^\circ$ (c 0.8 in *sym*-tetrachloroethane) and did not depress the melting point of authentic (–)-3',4',7-trimethoxy-2,3-*trans*-flavan-3,4-*trans*-diol [trimethyl ether of (+)-mollisacacidin]. Acetylation of the trimethyl ether with acetic anhydride–pyridine gave the diacetate, m.p. 104–105° undepressed by admixture of the authentic (+)-2,3-*trans*-3,4-*trans*-diacetate.

The optical rotations of the recovered (+)-mollisacacidin and its trimethyl ether, as well as the melting point of the trimethyl ether diacetate, showed that little or no racemization had occurred during heating (cf. Roux & Freudenberg, 1958; Clark-Lewis & Roux, 1959).

Some interconversions of the isomeric
2,3-*cis*-3,4-*cis*- and 2,3-*cis*-3,4-*trans*-diols

Conversion of the cis-cis diol into the cis-trans diol. The crystalline 3',4',7-trihydroxy-2,3-*cis*-flavan-3,4-*cis*-diol (1.5 mg.), m.p. 146–150°, R_F 0.41 in 2% (v/v) acetic acid and R_F 0.50 in water-satd. butan-2-ol, was heated on the water bath in 0.01 N-HCl (0.5 ml.) for 10 min. Two-dimensional chromatography of the product showed an approx. 3:2 mixture of the starting material and of the *cis-trans* isomer [R_F 0.51 in 2% (v/v) acetic acid and R_F 0.60 in water-satd. butan-2-ol].

Conversion of the cis-trans diol into the 4-ethyl ether. The *cis-trans*-diol, purified by chromatography, was refluxed for 1.5 hr. with ethanol (1 ml.) containing 1% (v/v) of acetic acid. Two-dimensional paper chromatography showed approx. 50% conversion into the 4-ethyl ether [R_F 0.62 in 2% (v/v) acetic acid and R_F 0.84 in water-satd. butan-2-ol].

Conversion of the 4-ethyl ether into the cis-trans diol. The 4-ethyl ether of the *cis-trans*-diol (2 mg.), m.p. 187–188°, was heated for 20 min. at 97° (steam bath) with 0.1 N-acetic acid (0.5 ml.). Two-dimensional chromatography of the products showed approx. 90% conversion into the free diol, R_F 0.51 and 0.61 in the above solvents respectively. No side reactions were observed other than the formation of traces of the *cis-cis* diastereoisomer.

Paper-electrophoretic examination of the
epimerization products

After epimerization of (+)-mollisacacidin (500 mg.) in 50 ml. of water, a 10 ml. sample was withdrawn and extracted with ethyl acetate, and the extract dried over Na₂SO₄. The dried extract was concentrated to dryness, dissolved in methanol and methylated with ethereal diazomethane at 0°. The product was examined by paper

electrophoresis in sodium borate in comparison with the synthetic racemates (Drewes & Roux, 1964b). The main band with migration -2.10 cm. was broad and consisted of a mixture of the 2,3-*trans*-3,4-*trans* and 2,3-*cis*-3,4-*trans* diastereoisomers. A band of intermediate concentration with migration $+3.2$ cm. represented the 2,3-*trans*-3,4-*cis* isomer, and the band of lowest concentration with migration $+1.6$ cm. was the 2,3-*cis*-3,4-*cis* isomer.

Confirmation of these allocations was possible after the isolation of individual products of epimerization and formation of their trimethyl ethers. The intensities of the bands were also in accordance with their concentrations in the mixture, as judged from their yields on isolation.

The following mobilities were observed for the methyl ethers of the various diastereoisomers: 2,3-*trans*-3,4-*trans* isomer, -2.10 cm.; 2,3-*trans*-3,4-*cis* isomer, $+3.20$ cm.; 2,3-*cis*-3,4-*trans* isomer, -2.05 cm.; 2,3-*cis*-3,4-*cis* isomer, $+1.70$ cm.

Anthocyanidin formation of isomeric flavan-3,4-diols

The four diastereoisomers were heated with propan-2-ol-3 N-HCl (4:1, v/v) on the water bath for 1.5 hr. (Pigman, Anderson, Fischer, Buchanan & Browning, 1953). Paper chromatography showed that 3,3',4',7-tetrahydroxyflavylum chloride (fisetinidin chloride), R_F 0.43 in 3 N-HCl-formic acid (1:1, v/v) (Roux, 1957), λ_{max} 525 m μ , was formed in all cases.

The yields of fisetinidin chloride were estimated (cf. Roux & Evelyn, 1958): from the 2,3-*trans*-3,4-*trans* isomer, 21.6 and 23.3% yield; from the 2,3-*trans*-3,4-*cis* isomer, 15.5 and 14.4% yield; from the 2,3-*cis*-3,4-*trans* isomer, 26.4 and 27.4% yield; from the 2,3-*cis*-3,4-*cis* isomer, 30.1 and 30.9% yield.

Epimerization reactions of isomelacacidin and (-)-melacacidin

(-)-Melacacidin and isomelacacidin (Clark-Lewis & Mortimer, 1960) were isolated from the heartwood of *Acacia melanoxylon* as described by Drewes & Roux (1964b). Each compound (15 mg.) was dissolved in water (5 ml.) and autoclaved in the domestic pressure cooker under the conditions used for (+)-mollisacacidin. Two-dimensional paper chromatography of the products showed that conversion of the 2,3-*cis*-3,4-*cis*-diol [(-)-melacacidin] into the 2,3-*cis*-3,4-*trans*-diol (isomelacacidin) proceeded (approx. 50–55% conversion) readily under these conditions.

The reverse conversion of isomelacacidin into (-)-melacacidin proceeds to a smaller extent (approx. 15–20% conversion), and it appears that an equilibrium might be established between these stereoisomeric forms.

DISCUSSION

The epimerization of (+)-mollisacacidin [(+)-3',4',7-trihydroxy-2,3-*trans*-flavan-3,4-*trans*-diol] by autoclaving in aqueous solution generates three optically active diastereoisomers. These are separable by preparative paper chromatography and were isolated in the yields: 2,3-*cis*-3,4-*cis* (2.8%), 2,3-*cis*-3,4-*trans* (11.0%) and 2,3-*trans*-3,4-*cis* (12.0%); 28.0% of unchanged starting material was

Table 1. Nuclear-magnetic-resonance spectra of diastereoisomeric 3,4-diacetoxy-3',4',7-trimethoxyflavans

	Chemical shifts: τ values (p.p.m.)									
	Me (acetyl)					Me (methoxyl)				
	3-	4-	3'-	4'-	7-	2-	3-	4-	5-	H
(+)-2,3- <i>trans</i> -3,4- <i>trans</i> (I)*	8.15 (8.15)	7.97 (7.97)	6.24 (6.23)	6.12 (6.12)	6.09 (6.10)	4.94 (4.95)	4.42 (4.42)	3.74 (3.78)	~2.92 (~2.92)	
(+)-2,3- <i>trans</i> -3,4- <i>cis</i> (II)*	8.16 (8.15)	7.87 (7.87)	6.24 (6.22)	6.10 (6.10)	6.09 (6.12)	4.79 (4.80)	4.49 (4.47)	3.82 (3.82)	2.77 (2.80)	
(+)-2,3- <i>cis</i> -3,4- <i>trans</i> (III)†	8.11 (8.11)	7.87 (7.86)	6.20	6.09 (6.10)	6.09 (6.10)	~4.72 (~4.80)	~4.72 (~4.80)	4.13 (4.16)	2.60	
(+)-2,3- <i>cis</i> -3,4- <i>cis</i> (IV)*	8.05 (8.06)	7.88 (7.88)	6.19 (6.16)	6.09 (6.10)	6.09 (6.10)	4.69 (4.68)	4.33 (4.32)	3.67 (3.64)	~2.83	
Spin-spin coupling constants for 2-, 3- and 4-protons (cyc./sec.)										
	$J_{3,4}$					$J_{3,4}$				
(+)-2,3- <i>trans</i> -3,4- <i>trans</i> (I)*	8.9 (8.9)					7.1 (7.1)				
(+)-2,3- <i>trans</i> -3,4- <i>cis</i> (II)*	10.0 (10.3)					3.4 (3.3)				
(+)-2,3- <i>cis</i> -3,4- <i>trans</i> (III)†	<1.0 (1.0)					2.7 (~1.0)†				
(+)-2,3- <i>cis</i> -3,4- <i>cis</i> (IV)*	0.9 (~1.0)					4.4 (3.8, 4.2)				

* Values in parentheses are for the corresponding synthetic racemates (Drewes & Roux, 1964a).

† Values in parentheses are for 3,4-*trans*-diacetoxy-3',4'-dimethoxy-6-methyl-2,3-*cis*-flavan (Clark-Lewis *et al.* 1962).

‡ The published value of $J_{3,4}$ ~ 1.0 cyc./sec. for this compound is not accurate (Professor J. W. Clark-Lewis, personal communication).

recovered. Paper chromatograms indicated that the reaction conditions apparently allow few side reactions, and the total of 50% recovery must therefore be attributed mainly to losses incurred during repeated partition and adsorptive separations on cellulose.

The relative configurations of the optically pure 2,3-*cis*-3,4-*cis* and 2,3-*trans*-3,4-*cis* diastereoisomers were established by the identity of the nuclear-magnetic-resonance spectra of their trimethyl-ether diacetates with those of the corresponding synthetic racemates (Clark-Lewis & Williams, 1963; Drewes & Roux, 1964*a,b*) (cf. Table 1). However, the isomeric 2,3-*cis*-3,4-*trans* racemate has hitherto proved to be inaccessible, owing to difficulties latent in the synthesis of those flavan-3,4-diols of this configuration (Joshi & Kulkarni, 1957; Clark-Lewis, Jackman & Williams, 1962) that contain 5- (Chandorkar, Phatak & Kulkarni, 1962) or 7-methoxyl substituents. For the optically pure (+)-2,3-*cis*-3,4-*trans* diastereoisomer, the spin-spin coupling constants of the 2-, 3- and 4-protons in the heterocyclic ring system of the same derivative, as well as the chemical shifts of these protons, are in close agreement (cf. Table 1) with those obtained for ($\pm$)-3,4-*trans*-diacetoxy-3',4'-dimethoxy-6-methyl-2,3-*cis*-flavan (Clark-Lewis *et al.* 1962). The nuclear-magnetic-resonance spectra of these 2,3-*cis*-3,4-*trans*-diacetates and of ($\pm$)-3,4-

trans-diacetoxy-4'-methoxy-6-methyl-2,3-*cis*-flavan (portion of the spectrum of the latter was kindly supplied by Professor J. W. Clark-Lewis) are characterized by the unique overlap of 2- and 3-protons, and the upfield position of the 4-protons (τ 4.13–4.16 p.p.m.) compared with those of all other stereoisomers (τ 3.69–3.84 p.p.m.).

Confirmation of these relative configurations for the diastereoisomeric flavan-3,4-diols from (+)-mollisacacidin is provided by the paper-electrophoretic mobilities of their trimethyl ethers in sodium borate. Thus the degree of anodic mobility of the 2,3-*cis*-3,4-*cis* and 2,3-*trans*-3,4-*cis* diastereoisomers are in agreement with values found for the corresponding synthetic racemates by Drewes & Roux (1964*b*), and the apparent cathodic mobilities (presumably due to electro-endosmosis) of the 2,3-*cis*-3,4-*trans* and recovered 2,3-*trans*-3,4-*trans* diastereoisomers are similar, as expected. Also, the ease of formation, and of hydrolysis, of the 4-ethyl ether of the 2,3-*cis*-3,4-*trans* diastereoisomer, and the ready inversion at C-4 in aqueous 0.01*N*-hydrochloric acid of the 2,3-*cis*-3,4-*cis* to give the 2,3-*cis*-3,4-*trans* diastereoisomer, parallels similar conversions of isomelacacidin and (–)-melacacidin (Clark-Lewis & Mortimer 1960) which have similarly related relative configurations.

Recovery of (+)-mollisacacidin from the reaction

mixture with little or no indication of racemization under conditions used suggests that the three diastereoisomers formed are likely to be optically pure. Their movement as discrete spots on paper chromatograms in 2% (v/v) acetic acid supports this view.

Considerations of the relative configurations of the diastereoisomers, their high optical activity, the ready epimerization of flavan-3-ols (Freudenberg & Purrmann, 1923; Roberts & Wood, 1953; Weinges, 1964) and flavan-3,4-diols (Clark-Lewis & Mortimer, 1960; Kashikar & Kulkarni, 1961; Fujise *et al.* 1962; Drewes & Roux, 1964a) at C-2 and C-4 respectively, and the premise that no inversion occurs at C-3 under such conditions (Roberts & Wood, 1953; Freudenberg, 1956), suggest a scheme of inter-conversion as shown. Thus the 2,3-*trans*-3,4-*cis* (II) and 2,3-*cis*-3,4-*trans* (III) diastereoisomers are formed by epimerization of the (+)-2,3-*trans*-3,4-*trans*-diol [(+)-mollisacacidin] at C-4 and C-2 respectively. Each of these may undergo further epimerization at C-2 and C-4 respectively to form the 2,3-*cis*-3,4-*cis* (IV) diastereoisomer. The proportions of these diastereoisomers occurring in the reaction mixture may represent an equilibrium set up under the conditions of autoclaving. These notions are supported by the ready conversion of (–)-melacacidin (3',4',7,8-tetrahydroxy-2,3-*cis*-flavan-3,4-*cis*-diol) into isomelacacidin (the corresponding 2,3-*cis*-3,4-*trans* diastereoisomer), but a lower percentage reverse conversion under identical conditions in the autoclave.

From the above, and from the known absolute configuration of (+)-mollisacacidin (I) (Clark-Lewis & Williams, 1963; Drewes & Roux, 1964a), the absolute configurations of the diastereoisomeric flavan-3,4-diols may be assigned as follows: (I) (2,3-*trans*-3,4-*trans* isomer), 2*R*:3*S*:4*R*; (II) (2,3-*trans*-3,4-*cis* isomer), 2*R*:3*S*:4*S*; (III) (2,3-*cis*-3,4-*trans* isomer), 2*S*:3*S*:4*R*; (IV) (2,3-*cis*-3,4-*cis* isomer), 2*S*:3*S*:4*S*. Epimerization of (+)-mollisacacidin thus furnishes the free phenolic forms of a set of four related (at C-3) diastereoisomers for the first time. These have assisted the identification of some of their natural enantiomers (Drewes & Roux, 1964e).

The relative ease of epimerization of (+)-mollisacacidin re-emphasizes the careful handling required during the isolation of associated 'tannin' polymers from the bark and heartwood of *A. mearnsii* (Roux & Paulus, 1962; Drewes & Roux, 1963). Similar epimerization of polymeric flavan-3,4-diols may be partly responsible for the apparent increase in complexity of wattle-bark tannins (mainly polymeric leuco-robinetinidins and leuco-fisetinidins) after the prolonged heating during the extraction and vacuum-evaporation stages of commercial extraction.

Free phenolic forms of the diastereoisomeric flavan-3,4-diols all darken on pyrolysis (heating to melting or decomposition point) (cf. Drewes & Roux, 1964c) with more intense reddening observable in the 2,3-*trans*-3,4-*trans* and 2,3-*cis*-3,4-*cis* than in the 2,3-*trans*-3,4-*cis* and 2,3-*cis*-3,4-*trans* arrangements. This suggests that configurational factors in the 3- and 4-positions may affect the degree of reddening under pyrolytic conditions, as in the comparison of peltogynol B with peltogynol (Chan, Forsyth & Hassall, 1958). Similarly anthocyanidin formation in the presence of mineral acid, due to the loss of the elements of water from the 3- and 4-positions, appears to be partly affected by the stereochemistry of the flavan-3,4-diol grouping, although the yields of anthocyanidin require confirmation from other sets of diastereoisomers.

This work is supported by the annual grant of the African Territories Wattle Industry Fund to the Leather Industries Research Institute.

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