- Buchanan, J. G., Dekker, C. A. & Long, A. G. (1950). *J. chem. Soc.* p. 3102.
- Chen, P. S., Toribara, T. Y. & Warner, H. (1956). *Analyt. Chem.* **28**, 1756.
- Consden, R. & Gordon, A. H. (1948). *Nature, Lond.*, **162**, 180.
- Critchley, P., Archibald, A. R. & Baddiley, J. (1962). *Biochem. J.* **85**, 420.
- Davison, A. L. (1962). Ph.D. Thesis: University of Durham.
- Dixon, J. S. & Lipkin, D. (1954). *Analyt. Chem.* **26**, 1092.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). *Analyt. Chem.* **28**, 350.
- Ellwood, D. C., Kelemen, M. V. & Baddiley, J. (1963). *Biochem. J.* **86**, 213.
- Fischer, F. G. & Nebel, H. G. (1955). *Hoppe-Seyl. Z.* **302**, 10.
- Foster, A. B., Horton, D. & Stacey, M. (1957). *J. chem. Soc.* p. 81.
- Goldstein, I. J. & Whelan, W. J. (1962). *J. chem. Soc.* p. 170.
- Hanahan, D. J. & Olley, J. N. (1958). *J. biol. Chem.* **231**, 813.
- Hanes, C. S. & Isherwood, F. A. (1949). *Nature, Lond.*, **164**, 1107.
- Haukenes, G. (1962). *Immunochemical Studies on Polysaccharide A of Staphylococcus aureus*. Norwegian Universities Press.
- Haukenes, G., Ellwood, D. C., Baddiley, J. & Oeding, P. (1961). *Biochim. biophys. Acta*, **53**, 425.
- Hay, J. B., Wicken, A. J. & Baddiley, J. (1963). *Biochim. biophys. Acta* (in the Press).
- Hough, L., Powell, D. B. & Woods, B. M. (1956). *J. chem. Soc.* p. 4799.
- Kelemen, M. V. & Baddiley, J. (1961). *Biochem. J.* **80**, 246.
- Morse, S. I. (1962). *J. exp. Med.* **116**, 229.
- Rosen, H. (1957). *Arch. Biochem. Biophys.* **67**, 10.
- Sanderson, A. R., Juergens, W. G. & Strominger, J. L. (1961). *Biochem. biophys. Res. Commun.* **5**, 472.
- Trevelyan, W. E., Procter, D. P. & Harrison, J. S. (1950). *Nature, Lond.*, **166**, 444.
- Wicken, A. J. & Baddiley, J. (1963). *Biochem. J.* **87**, 54.
- Wicken, A. J., Elliott, S. D. & Baddiley, J. (1963). *J. gen. Microbiol.* **30**, 111.

Biochem. J. (1963) **87**, 435

Condensed Tannins

16. SYNTHESIS OF (–)-FLAVANS FROM (+)-DIHYDROFLAVONOLS*

By D. G. ROUX†

Leather Industries Research Institute, Rhodes University, Grahamstown, South Africa

(Received 18 July 1962)

By means of the reductive conversions of (+)-dihydorobinetin and (+)-fustin [(+)-3',4',5',7-tetrahydroxy- and (+)-3',4',7-trihydroxy-flavanon-3-ol respectively] into their (–)-flavanone and thence into their (+)-flavan-4-ol analogues, Roux & Paulus (1962*b*) were able to correlate the absolute configurations of each of these classes of flavonoid compounds. Similarly, the absolute configurations of the corresponding (–)-flavans may now be predicted by an extension of the same series of interconversions.

From the above and also from previous work on their naturally occurring analogues, the contributions of each of the asymmetric centres to the molecular rotations of natural flavan-3-ols, flavan-3,4-diols, flavanonols and synthetic flavan-4-ols, flavans and flavanones may be assessed. Also, evidence from the conversion of flavan-4-ols into flavans confirms previous notions (Roux & Paulus, 1962*b*) on the contribution of the 4-hydroxyl group to the redness induced in tannins by sunlight.

* Part 15: Drewes & Roux (1963).

† Present address: Chemistry Department, Harvard University, Cambridge 38, Mass., U.S.A.

EXPERIMENTAL AND RESULTS

All melting points are uncorrected. Mixed melting points were carried out as described by Roux & Maihs (1960*a*). Analysis for C, H, methoxyl and acetyl groups was by K. Jones, Microanalytical Laboratory, C.S.I.R., Pretoria, South Africa. Comparisons of infrared spectra were by Dr P. R. Enslin, National Chemical Research Laboratories, C.S.I.R., Pretoria, South Africa. A 2 dm. tube was used for optical rotations.

Synthesis of (±)-3',4',5',7-tetrahydroxyflavan and derivatives from (±)-dihydorobinetin

Partially racemized dihydorobinetin, $[\alpha]_D +16^\circ$, from the heartwood of *Robinia pseudacacia* (Freudenberg & Hartmann, 1954) was separated into a racemate and into the optically pure (+)-form by chromatographic methods with cellulose as substrate (Roux & Paulus, 1962*b*).

(±)-3',4',5',7-Tetrahydroxyflavan. Racemic dihydorobinetin (500 mg.) was selectively reduced to (±)-robinetin [(±)-3',4',5',7-tetrahydroxyflavanone] (250 mg.), m.p. 276°, with deactivated zinc dust and hydrochloric acid in methanolic solution (Roux & Paulus, 1962*b*). The method of reduction was improved by using AnalaR zinc dust that had been dampened and left in an open beaker at room temperature (20–30°) for 20 days. The zinc set into a hard

grey-white mass which was finely powdered before use. Improved yields were also obtained by a uniform rate of addition of the conc. hydrochloric acid (2 drops/2 min. for 1.5 hr., then 1 drop/2 min. for the final 0.5 hr.) to the powdered zinc in the methanolic solution of the dihydroflavonol. The yield of ($\pm$)-robtin (200–250 mg.) from 500 mg. of ($\pm$)-dihydorobinetin was invariably less than that of ($\pm$)-robtin (300–340 mg.) from the same weight of optically pure (+)-dihydorobinetin.

($\pm$)-Robtin (600 mg.) was hydrogenated with palladium (300 mg. of palladium chloride) in dioxan solution in the cold for 10.5 hr., during which time 157 ml. of hydrogen was absorbed. The hydrogenation was discontinued at this stage although hydrogen was still being taken up (at a much diminished rate). The same dioxan solution was evaporated under reduced pressure, water (15 ml.) was added to the oily residue and the solution was concentrated to small volume (5 ml.) to expel traces of dioxan. The product crystallized readily in small stout needles, which were recrystallized from water, m.p. 205–206° (Found: C, 65.7; H, 5.2. $C_{15}H_{14}O_6$ requires C, 65.7; H, 5.1%). It had R_F 0.25 in 2% (v/v) acetic acid, and R_F 0.72 in water-saturated butan-1-ol. The flavan gave the colours indicated with the following spray reagents (cf. Roux & Maihs, 1960b): ferric alum (blue), bis-diazotized benzidine (deep yellow), ammoniacal silver nitrate (black or brown), quinonechloroimide followed by fuming with ammonia (brown), 2,6-dichloroquinonechloroimide (mauve fading to brown) and 2,6-dibromoquinonechloroimide (mauve edges with bright-yellow centre). The substance showed no tendency towards redness on drying or on storage. The infrared spectrum (potassium bromide disk) showed the absence of the carbonyl absorption band that is present in the spectrum of robtin at 1640 cm^{-1} .

($\pm$)-3',4',5',7-Tetramethoxyflavan. ($\pm$)-3',4',5',7-Tetrahydroxyflavan (100 mg.) was methylated with diazomethane in methanolic solution as described by Roux & Paulus (1960) to give 39 mg. of fine needles from ethanol after recrystallization, m.p. 92–93° (Found: C, 68.7; H, 6.6; $O \cdot CH_3$, 37.1. $C_{19}H_{22}O_8$ requires C, 69.1; H, 6.7; $O \cdot CH_3$, 37.6%). The infrared spectrum showed the absence of carbonyl absorption over the range 1725–1640 cm^{-1} .

($\pm$)-3',4',5',7-Tetra-acetoxyflavan. ($\pm$)-3',4',5',7-Tetrahydroxyflavan (75 mg.) was acetylated with acetic anhydride and pyridine as described by Roux & Paulus (1960) to give 70 mg. of fine needles from ethanol, m.p. 90–93° (Found: C, 62.3; H, 5.2; $CO \cdot CH_3$, 37.4. $C_{23}H_{22}O_9$ requires C, 62.4; H, 5.0; $CO \cdot CH_3$, 38.9%).

Synthesis of ($\pm$)-3',4',5',7-Tetrahydroxyflavan from ($\pm$)-3',4',5',7-tetrahydroxyflavan-4-ol

($\pm$)-3',4',5',7-Tetrahydroxyflavan-4-ol (300 mg.) (Roux & Paulus, 1962b) which had developed a red-blue on exposure to indirect sunlight was hydrogenated with palladium as described above to give ($\pm$)-3',4',5',7-tetrahydroxyflavan (93 mg.), m.p. and mixed m.p. with the above 204–206°. The flavan retained its slightly buff appearance on storage and exposure to indirect sunlight.

Synthesis of ($\pm$)-3',4',5',7-tetrahydroxyflavan from (+)-dihydorobinetin

By using methods described above, 500 mg. of (+)-dihydorobinetin, $[\alpha]_D +27.8^\circ$ [c 1.0 in acetone-water (1:1,

v/v)] was converted into ($\pm$)-robtin, m.p. 276°, $[\alpha]_D^{25} -56.8 \pm 0.8^\circ$ (c 0.9 in methanol) and $-32.4 \pm 0.4^\circ$ [c 1.0 in acetone-water (1:1, v/v)]. ($\pm$)-Robtin (600 mg.) was hydrogenated with palladium in dioxan as described above to give 340 mg. of short stout needles of ($\pm$)-3',4',5',7-tetrahydroxyflavan from water, m.p. and mixed m.p. with ($\pm$)-3',4',5',7-tetrahydroxyflavan 207–209°, $[\alpha]_D^{25} -35.5 \pm 0.5^\circ$ in (c 1.1 in methanol) and $-20.0 \pm 0.6^\circ$ [c 1.0 in acetone-water (1:1, v/v)].

Synthesis of ($\pm$)-3',4',7-trihydroxyflavan and derivatives from ($\pm$)-fustin

($\pm$)-3',4',7-Trihydroxyflavan. ($\pm$)-Fustin, m.p. 226–228°, from *Rhus glabra* (Roux & Paulus, 1960) was converted into ($\pm$)-butin, m.p. 223–225°, by reduction with deactivated zinc dust and hydrochloric acid in methanolic solution (Roux & Paulus, 1962b). ($\pm$)-Butin (950 mg.) was hydrogenated with palladium (500 mg. of palladium chloride) in dioxan solution, 310 ml. of hydrogen being absorbed over 10 hr. The reaction was discontinued although hydrogen absorption was still proceeding. The product was sparingly soluble in water, from which it settled as an oil which crystallized at 0° over 48 hr., m.p. 185–187°. White crystals were obtained from water on recrystallization, m.p. 188–190° (Found: C, 69.7; H, 5.6. $C_{15}H_{14}O_4$ requires C, 69.8; H, 5.5%). The compound migrated as a single spot on two-dimensional chromatograms run successively in water-saturated butan-1-ol (R_F 0.80) and 2% (v/v) acetic acid (R_F 0.27). The flavan gave the following colours with the spray reagents (cf. Roux & Maihs, 1960b): ferric alum (olive-green), bis-diazotized benzidine (yellow), silver nitrate (grey-black), quinonechloroimide followed by fuming with ammonia (weak violet fading to buff), 2,6-dichloroquinonechloroimide (deep blue fading rapidly to a mauve-brown) and 2,6-dibromoquinonechloroimide (weak powder-blue fading to buff). The crystals remained white on heating and on aging. The infrared spectrum (potassium bromide disk) showed the absence of the carbonyl absorption band which is present in the spectrum of butin at 1650 cm^{-1} .

($\pm$)-3',4',7-Triacetoxyflavan. The acetate was formed with acetic anhydride and pyridine as described above, and crystallized in needles from ethanol, in which it was sparingly soluble, m.p. 140–141° after sintering at 90° (Found C, 65.4; H, 5.3; $CO \cdot CH_3$, 33.1. $C_{21}H_{20}O_7$ requires C, 65.6; H, 5.2; $CO \cdot CH_3$, 33.6%).

Methyl derivative of ($\pm$)-3',4',7-trihydroxyflavan. ($\pm$)-3',4',7-Trihydroxyflavan gave an oily methylated derivative after methylation with diazomethane. The product failed to harden at -15° and was not analysed.

Synthesis of ($\pm$)-3',4',7-trihydroxyflavan from (+)-fustin

Fustin, m.p. 228–229°, $[\alpha]_D^{25} +28.3 \pm 0.6^\circ$ [c 1.0 in acetone-water (1:1, v/v)], from the heartwood of *Acacia mearnsii* (*A. mollissima*) (Roux & Paulus, 1960), was reduced with deactivated zinc and hydrochloric acid to ($\pm$)-butin, m.p. 225–227°, $[\alpha]_D^{25} -59.8 \pm 1.0^\circ$ (c 0.5 in methanol) and $-33.6 \pm 0.4^\circ$ [c 0.8 in acetone-water (1:1, v/v)] (Roux & Paulus, 1962b). The optically pure ($\pm$)-butin (300 mg.) was hydrogenated with palladium (200 mg. of palladium chloride) in dioxan as described above to give ($\pm$)-3',4',7-trihydroxyflavan, m.p. and mixed m.p. with

($\pm$)-3',4',7-trihydroxyflavan 188–190°, $[\alpha]_D^{21} - 34.1 \pm 0.3^\circ$ (*c* 0.6 in methanol) and $-18.5 \pm 0.3^\circ$ [*c* 1.0 in acetone–water (1:1, *v/v*)].

DISCUSSION

The conversion of dihydroflavonols into flavanones by selective reduction with zinc and hydrochloric acid, and thence into flavans by catalytic reduction with palladium, provides an easy method of partial synthesis of flavans. Although a similar conversion of the unsubstituted parent flavanone into flavan, by using lithium aluminium hydride, was described by Bokadia, Brown, Coburn, Roberts & Somerfield (1962), the catalytic reduction of flavanones containing a hydroxyl group in the 4'-position results in ring opening. Thus naringenin (4',5,7-trihydroxyflavanone) is slowly reduced with palladium in ethanolic solution with the absorption of 1 mole of hydrogen/mole to give phloretin [3-(*p*-hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one] (Rosenmund & Rosenmund, 1928). In naringenin the 4-carbonyl group is presumably protected from hydrogenation by the strong hydrogen bond of the 5-hydroxyl group, whereas in robtin and butin (5-deoxyflavanones) the unbonded carbonyl groups are preferentially reduced. Both robtin and fustin, however, have hydroxyl groups in the 4'-position as in naringenin and their reduction with palladium shows evidence of proceeding further with heterocyclic ring opening (splitting of a *p*-hydroxybenzyl ether link), resulting probably in substituted 1,3-diphenylpropanes. The retention of optical activity in the flavans, as well as their analyses, show that reduction had been discontinued before cleavage of the heterocyclic ring.

Conversion of (+)-dihydrorobinetin [(2*R*:3*R*)-3,3',4',5',7-pentahydroxyflavan-4-one] and (+)-fustin [(2*R*:3*R*)-3,3',4',7-tetrahydroxyflavan-4-one] (Weinges, 1958; Roux & Paulus, 1960) into (-)-robtin [(2*S*)-3',4',5',7-tetrahydroxyflavan-4-

one] (I, R = OH) and (-)-butin [(2*S*)-3',4',7-trihydroxyflavan-4-one] (I, R = H) respectively (Roux & Paulus, 1962*b*), and thence into (-)-3',4',5',7-tetrahydroxyflavan (II, R = OH) and (-)-3',4',7-trihydroxyflavan (II, R = H), establishes the absolute configuration of both flavans as (2*S*), the 2-phenyl group occupying the same relative position as in (+)-catechin.

(I) R = OH, (-)-Robtin;
R = H, (-)-butin

(II) R = OH, (-)-3',4',5',7-Tetrahydroxyflavan; R = H, (-)-3',4',7-trihydroxyflavan

The series of interconversions: (+)-dihydroflavonols $\rightarrow$ (-)-flavanones $\rightarrow$ (+)-flavan-4-ols $\rightarrow$ (-)-flavans, therefore, not only establish the absolute configurations of the flavanones and flavans and the configuration of flavan-4-ols at C-2, but also the contributions at each of the asymmetric centres C-2 and C-3 to the molecular rotations of these as well as of natural analogues (Tables 1 and 2). For example, the molecular rotations of the flavans (II, R = H and OH) in acetone–water (-55° and -48° respectively) indicate the negative contribution of the (2*S*)-asymmetric carbon, corresponding to (2*R*) in the

Table 1. *Specific and molecular rotations of analogues of (+)-leuco-robinetinidin and (+)-leuco-fisetinidin in acetone–water (1:1, *v/v*)*

For specific rotations of flavan-3-ols see Weinges (1958), Roux & Maihs (1960*a*), Roux & Paulus (1961); of flavan-4-ols see Roux & Paulus (1962*b*); of flavan-3,4-diols see Weinges (1958), Roux & Paulus (1962*a*) and Clark-Lewis & Roux (1959); and of flavanonols see Weinges (1958), Roux & Paulus (1960, 1962*b*).

	3',4',5',7-Tetrahydroxy compounds		3',4',7-Trihydroxy compounds	
	$[\alpha]_D$	$[M]_D$	$[\alpha]_D$	$[M]_D$
(a) (-)-Flavan	-20.0°	-55°	-18.5°	-48°
(b) (-)-Flavan-3-ol	{ -10.7 -11.5 }	-31	{ -10.1 -8.6 }	-28
(c) (+)-Flavan-4-ol	+24.2	+70	+24.9	+72
(d) (+)-Flavan-3,4-diol	+33.9	+104	+32.0	+93
(e) (-)-Flavanone	-32.4	-93	-33.6	-91
(f) (+)-Flavanonol	{ +27.8 +29 }	+85	+28.3	+82

Table 2. Contributions to molecular rotations of flavonoids of the 'resorcinol series' at the asymmetric centres C-2, C-3 and C-4

The configurations of the substituents at C-2 and C-3 are identical for all compounds in Table 1. The identification and their differences relate to the sequence used in Table 1.

	3',4',5',7-Tetrahydroxy compounds [M] _D	3',4',7-Trihydroxy compounds [M] _D
Contribution at C-2		
Flavans (a)	- 55°	- 48°
Flavanones (e)	- 93	- 91
Contribution at C-3: addition of 3-hydroxy group to		
Flavans (b) - (a)	+ 24	+ 20
Flavan-4-ols (d) - (c)	+ 34	+ 21
Flavanones (f) - (e)	+ 178	+ 173
Contribution at C-4: addition of 4-hydroxy group to		
Flavans (c) × (a)	+ 125	+ 120
Flavan-3-ols (d) - (b)	+ 135	+ 117

flavan-3-ols and flavan-3,4-diols. Molecular-rotational differences between flavans and flavan-3-ols (+24° and +20°), and between flavan-4-ols and flavan-3,4-diols (+34° and +21°), determine the contribution made by the (3S)-asymmetric centre. Similarly, rotational differences between flavans and flavan-4-ols (+125° and +120°) and between flavan-3-ols and flavan-3,4-diols (+135° and +117°) demonstrate the large positive contribution at C-4. From agreements between these molecular-rotational differences summarized in Table 2, it appears that contributions of the individual asymmetric centres C-2 and C-3 to the molecular rotations of these analogues are additive. This points to the almost complete absence of vicinal action of asymmetric centres (Klyne, 1960) in these closely related flavans, and the data available might, in conjunction with other evidence, assist in determining the absolute configurations of new analogues of the same conformation and identical pattern of phenolic hydroxylation. Thus (+)-flavan-4-ols and (+)-flavan-3,4-diols formed by catalytic reduction of (-)-flavanones and (+)-dihydroflavanols respectively (Weinges, 1958; Roux & Paulus, 1960, 1962b) should have the same configuration of the hydroxyl group at C-4 owing to similar contributions to the molecular rotations in both classes of compounds (cf. Table 2). At present this configuration is tentatively regarded as (4R) for the flavan-4-ols, corresponding to (4S) for the flavan-3,4-diols, and is based on the formation of isopropylidene derivatives of the latter in good yield with consequent assignment of a *cis*-3,4-diol structure (Clark-Lewis & Roux, 1959; Freudenberg & Weinges, 1959; Clark-Lewis & Katekar, 1960; Roux & Paulus, 1962a, b).

However, from examination of specific rotations alone, it appears that substitution of an hydroxyl group in the 5-position, as in the 'phloroglucinol series', alters the magnitude of the contribution at some asymmetric centres but without alteration in the direction of rotation. Thus, by assuming the absence of vicinal action also in (+)-catechin [(2R:3S)-3',4',5,7-tetrahydroxyflavan-3-ol], [α]_D + 17.1° ([M]_D + 49.6°) and (-)-epicatechin [(2R:3R)-3',4',5,7-tetrahydroxyflavan-3-ol], [α]_D - 60° ([M]_D - 174°) (Freudenberg & Purmann, 1923, 1924; Birch, Clark-Lewis & Robertson, 1957), the contributions of the asymmetric centres of the molecular rotations of these compounds may be calculated as -62° at C-2 and +112° at C-3. The calculated contribution at C-2 is almost the same as those found in the 'resorcinol series', but at C-3 the magnitude of the rotation differs in the two series (cf. Table 2). Also, introduction of a carbonyl group at C-4, as in the flavanonols, increases the magnitude of the contributions to the molecular rotations at C-2 and C-3 without altering their direction (cf. Table 2).

Hydrogenolysis of the flavan-4-ols removes the 4-hydroxyl group to form flavans with concomitant loss of the ability to develop redness on exposure of the solid product to direct or indirect sunlight. This proves unequivocally that the 4-hydroxyl group is contributory to the redness induced in flavan-4-ols, flavan-3,4-diols and polymeric flavan-3,4-diols (leuco-anthocyanidin tannins) under these conditions. This factor is of importance in the commercial use of condensed tannins.

SUMMARY

1. The absolute configurations [both (2S)] of (-)-3',4',5',7-tetrahydroxyflavan and (-)-3',4',7-trihydroxyflavan have been established by inter-conversions from dihydroflavanols and flavanones of known configuration.

2. The contributions to the molecular rotations of some flavan and flavanone analogues at each of the asymmetric centres C-2, C-3 and C-4 have been determined. These appear to be additive for flavan analogues of the 3',4',5',7 and 3',4',7 patterns of phenolic hydroxylation. This knowledge might assist in predicting the absolute configuration of analogues of the same series.

3. Proof has been provided that the 4-hydroxyl group is mainly contributory to the redness induced in flavonoid compounds by sunlight.

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

REFERENCES

- Birch, A. J., Clark-Lewis, J. W. & Robertson, A. V. (1957). *J. chem. Soc.* p. 3586.
- Bokadia, M. M., Brown, B. R., Coburn, D., Roberts, A. & Somerfield, G. A. (1962). *J. chem. Soc.* p. 1658.
- Clark-Lewis, J. W. & Katekar, G. F. (1960). *Proc. chem. Soc., Lond.*, p. 345.
- Clark-Lewis, J. W. & Roux, D. G. (1959). *J. chem. Soc.* p. 1402.
- Drewes, S. E. & Roux, D. G. (1963). *Biochem. J.* **87**, 167.
- Freudenberg, K. & Hartmann, L. (1954). *Liebigs Ann.* **587**, 207.
- Freudenberg, K. & Purrmann, L. (1923). *Ber. dtsh. chem. Ges.* **56** B, 1185.
- Freudenberg, K. & Purrmann, L. (1924). *Liebigs Ann.* **437**, 274.
- Freudenberg, K. & Weinges, K. (1959). *Chem. & Ind.* p. 486.
- Klyne, W. (1960). *Symp. biochem. Soc.* **19**, 17.
- Rosenmund, K. W. & Rosenmund, M. (1928). *Ber. dtsh. chem. Ges.* **61** B, 2608.
- Roux, D. G. & Maihs, A. E. (1960a). *Biochem. J.* **74**, 44.
- Roux, D. G. & Maihs, A. E. (1960b). *J. Chromat.* **4**, 65.
- Roux, D. G. & Paulus, E. (1960). *Biochem. J.* **77**, 315.
- Roux, D. G. & Paulus, E. (1961). *Biochem. J.* **78**, 120.
- Roux, D. G. & Paulus, E. (1962a). *Biochem. J.* **82**, 324.
- Roux, D. G. & Paulus, E. (1962b). *Biochem. J.* **84**, 416.
- Weinges, K. (1958). *Liebigs Ann.* **615**, 203.

Biochem. J. (1963) **87**, 439

Condensed Tannins

17. ISOLATION OF 4',7-DIHYDROXYFLAVAN-3,4-DIOL FROM *GUIBOURTIA COLEOSPERMA*

BY D. G. ROUX AND G. C. DE BRUYN

Leather Industries Research Institute, Rhodes University, Grahamstown, South Africa

(Received 3 September 1962)

Chromatographic evidence of the presence of a 4',7-dihydroxyflavan-3,4-diol, named 'guibourtao-acidin', in the heartwood of certain African *Guibourtia* spp. (Roux, 1959), previously *Copaifera* spp. (Léonard, 1949), also indicated that the compound probably was related to (+)-3',4',5',7-tetrahydroxyflavan-3,4-diol from *Robinia pseudacacia* (Weinges, 1958) and (+)-3',4',7-trihydroxyflavan-3,4-diol from *Acacia mearnsii* (Keppler, 1957). Thus it possibly formed a third member of these flavan-3,4-diols of the 'resorcinol series' with a tentatively assigned 2,3-*trans*-3,4-*cis* arrangement of substituent groups (Clark-Lewis & Roux, 1959; Roux & Paulus, 1962a). This possibility prompted Phatak & Kulkarni (1959) to synthesize a (±)-4',7-dimethoxyflavan-3,4-diol of the same configuration by reduction of the dihydroflavonol analogue.

The present work describes the isolation in very low yield of an optically active 4',7-dihydroxyflavan-3,4-diol from Rhodesian copalwood (*Guibourtia coleosperma*).

EXPERIMENTAL AND RESULTS

Two-dimensional chromatograms were run first in water-saturated butan-2-ol and then in 2% (v/v) acetic acid. Whatman no. 3 paper (22½ in. × 18½ in.) was used for preparative chromatography. Analyses of C, H, acetyl and methoxyl were by K. Jones, Microanalytical Laboratory,

C.S.I.R., Pretoria, South Africa, and infrared analyses were carried out under the supervision of Dr P. R. Enslin, National Chemical Research Laboratory, C.S.I.R., Pretoria, South Africa.

Enrichment procedure and isolation of leuco-anthocyanidins

Drillings (13 kg.) from the heartwood of *G. coleosperma* (Rhodesian copalwood) were covered with a commercial ethyl acetate-ethanol mixture (19:1, v/v) and the extract was evaporated to dryness under vacuum after extraction for 24 hr. Three successive extractions gave 404 g. of brown solids. These were extracted with light petroleum (b.p. 80–120°) and the wax-free solids were redissolved in a minimum of the same solvent mixture (1.6 l.), and an equal volume of chloroform was added with rapid stirring to precipitate the tannins (183 g.). The soluble fraction still contained material [R_F 0.0 in 2% (v/v) acetic acid, 0.90 in water-saturated butan-2-ol] that hindered preparative chromatographic separations in 2% acetic acid. This was precipitated by dissolving the tannin-free fraction in a minimum of hot ethanol and adding water (2.8 l.) slowly with shaking. Insoluble material (101 g.) was filtered off and the soluble fraction (120 g.) was recovered under vacuum.

The soluble residue was dissolved with warming in 400 ml. of the lower phase of water-butan-2-ol-light petroleum (b.p. 100–180°) (5:4:1, by vol.), and the mixture was filtered after cooling. The solution (60 g. of soluble material) was introduced into the first eight tubes of a 160-tube 50 ml.-underphase fully automatic Craig countercurrent machine (Glasapparatebau Göttingen-Helmut Rettberg).