galactose, and this was extracted from very young plants; sample 6 (Table 3) and sample 14 (Table 2) show low values of 3,6-anhydrogalactose, and both of these came from deteriorating plants, sample 6 from plants that were much bleached by insolation and sample 14 from old and tattered plants after a period of stormy weather. Correlation with the environment is suggested by Table 4, which shows the analyses of porphyran samples from collections of *P. perforata* made about the same time from a number of sites on the Pacific coast of North America. The molar percentages of 3,6-anhydrogalactose suggest that the greater the exposure the higher is the 3,6-anhydride content. Eppley & Cyrus (1960) have already suggested that one of the functions of porphyran might be to act as a cushion against physical buffeting; and it has been noticed in the present survey, as well as by Rees (1961a), that porphyran samples of high 3,6-anhydrogalactose content give thicker solutions and greater tendencies to form gels. Thus the amount of 3,6-anhydride present may be important as an adaptive ecological character.

SUMMARY

1. The water-soluble polysaccharides have been isolated and examined from about 40 different specimens of *Porphyra* species showing seasonal and environmental variations.

2. There is a wide variability in the composition of the polysaccharide extracts: 3,6-anhydro-L-galactose may be present in amounts from 5 to 19%, ester sulphate from 6 to 11%, 6-O-methyl-D-galactose from 3 to 28% and galactose from 24 to 45%.

3. Although the sulphate content is variable the

ester always seems to occur predominantly as 1→4-linked L-galactose 6-sulphate.

4. Regularities in the analytical results suggest that 3,6-anhydro-L-galactose and L-galactose 6-sulphate are interchangeable between the polysaccharides, and that D-galactose and 6-O-methyl-D-galactose are related in a similar manner.

5. In all the polysaccharides isolated, 3,6-anhydro-L-galactose and L-galactose 6-sulphate total approximately half of the sugar units, the other half being made up of D-galactose and 6-O-methyl-D-galactose.

6. The implications of these and other aspects of the results for the homogeneity, structure and biosynthesis of porphyran are discussed.

7. A preliminary attempt has been made to correlate polysaccharide composition with variation in the algal specimens examined.

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REFERENCES

- Eppley, R. W. & Cyrus, C. C. (1960). *Biol. Bull., Woods Hole*, **118**, 55.
 Frei, E. & Preston, R. D. (1961). *Nature, Lond.*, **192**, 931.
 Jones, A. S. & Letham, D. S. (1954). *Chem. & Ind.* p. 662.
 Peat, S. & Rees, D. A. (1961). *Biochem. J.* **79**, 7.
 Peat, S., Turvey, J. R. & Rees, D. A. (1961). *J. chem. Soc.* p. 1590.
 Rees, D. A. (1961a). *J. chem. Soc.* p. 5168.
 Rees, D. A. (1961b). *Biochem. J.* **81**, 347.
 Turvey, J. R. & Rees, D. A. (1961). *Nature, Lond.*, **189**, 831.
 Turvey, J. R. & Williams, T. P. (1961). *Proc. 4th. int. Seaweed Symp.* (in the Press).
 Wilson, C. M. (1959). *Analyt. Chem.* **31**, 1199.
 Yaphe, W. (1960). *Analyt. Chem.* **32**, 1327.

Biochem. J. (1962) **84**, 416

Condensed Tannins

14. FORMATION OF (−)-3',4',5',7-TETRAHYDROXYFLAVANONE AND (+)-3',4',5',7-TETRAHYDROXYFLAVAN-4-OL BY INTERCONVERSION FROM (+)-DIHYDROROBINETIN, AND SYNTHESIS OF THEIR RACEMATES*

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The isolation of the isomeric compounds (−)-robinin [(−)-3',4',5',7-tetrahydroxyflavanone] and robtein (2',3,4,4',5-pentahydroxychalcone) from

the heartwood of *Robinia pseudacacia* suggested to Roux & Paulus (1962) that they originate successively from (+)-dihydrorobinetin [(+)-3',4',5',7-tetrahydroxyflavanon-3-ol] as part of a more general scheme of flavonoid biogenesis based on the

* Part 13: Roux & Paulus (1962).

flavan-3,4-diol analogue. Synthesis of the chalcone and flavanone is described below, and the postulated conversion of (+)-dihydrorobinetin into (-)-robtin is effected *in vitro*. Further, (-)-robtin is converted into (+)-3',4',5',7-tetrahydroxyflavan-4-ol, a model substance for the study of the polymeric leucorobinetidin tannins present in black-wattle bark (*Acacia mearnsii*), and in the heartwood of the locust tree (*R. pseudacacia*). Parallel interconversions based on (+)-fustin [(+)-3',4',7-trihydroxyflavanon-3-ol] and of significance to wattle-heartwood tannins are also described.

These interconversions enable the absolute configurations of flavanonols, flavanones and flavan-4-ols to be correlated.

EXPERIMENTAL AND RESULTS

All melting points are uncorrected. Mixed melting points were carried out as described before (Roux & Maihs, 1960*a*). Analyses of C, H, methoxyl groups and acetyl groups were by K. Jones, Micro-analytical Section, National Chemical Research Laboratory, C.S.I.R., Pretoria. Infrared comparisons are by Dr J. R. Nunn of the same laboratories. A 2 dm. tube was used for optical rotations.

Synthesis of robtein, (±)-robtin and (±)-3',4',5',7-tetrahydroxyflavan-4-ol

Robtein (2',3,4,4',5-pentahydroxychalcone). Gallaldehyde, m.p. 210° (decomp.), was prepared by conversion of triacetyl gallic acid into triacetyl galloyl chloride by using thionyl chloride (Freudenberg & Hübner, 1952), followed by the hydrogenation of the last-named to triacetyl gallaldehyde (Rosenmund & Pfannkuch, 1922) with a 5% palladium-barium sulphate catalyst (Schmidt, 1919), and finally by hydrolysis of the triacetyl gallaldehyde with potassium acetate in ethanol (Rosenmund & Zetzsche, 1918).

Gallaldehyde (5 g.) and resacetophenone (5 g.), dissolved in ethanol (7 ml.), were refluxed under hydrogen with aqueous potassium hydroxide (50 g. in 35 ml.) for 30 min., and the dark solid that separated on cooling was left overnight under hydrogen. The reaction mixture was diluted with an equal volume of water, cooled in ice and acidified with dilute hydrochloric acid. The deep-yellow precipitate was recrystallized from ethanol-water to give yellow crystals (1.5 g.), which darken above 280° but do not melt below 345°. Shorter reaction periods give decreased yields.

The synthetic compound and robtein from *R. pseudacacia* (cf. Roux & Paulus, 1962) had the same R_F (0.68) on Whatman no. 1 paper in butan-1-ol-acetic acid-water (6:1:2, by vol.) (upward migration), and both became brick-red after treatment with ammonia vapour. The pentaacetate of the synthetic compound had m.p. 124–126°, undepressed (m.p. 124–125°) by admixture with pentaacetylrobtin (m.p. 124–126°) derived from *R. pseudacacia* (Roux & Paulus, 1962).

(±)-*Robtin* [(±)-3',4',5',7-tetrahydroxyflavanone]. Robtein (1 g.) in ethanol (3 ml.) was refluxed with 1.5% (w/v)

sodium hydroxide (10 ml.) for 30 min. in a current of hydrogen, and the mixture was left overnight under hydrogen at room temperature. The mixture was diluted with water (10–15 ml.) in a current of hydrogen and acidified with dilute acetic acid. Unchanged robtein that separated on acidification was removed. From the mother liquor 0.15 g. of pale-yellow crystals separated, m.p. 276° (decomp.) after sintering at 160–165°. Mixed m.p. with robtin (m.p. 276°) from *R. pseudacacia* (Roux & Paulus, 1962) showed no depression.

Synthetic (±)-robtin gave a tetra-acetate, white needles, m.p. and mixed m.p. with tetra-acetylrobtin from *R. pseudacacia*, 180–182°.

(±)-3',4',5',7-Tetrahydroxyflavan-4-ol. Hydrogenation of synthetic (±)-robtin (600 mg.) dissolved in methanol (50 ml.) in the presence of Adams platinum dioxide (300 mg.) gave (±)-3',4',5',7-tetrahydroxyflavan-4-ol (300 mg.) as white needles after recrystallization from water. These sintered slightly at 336° but did not melt (Found: C, 62.4; H, 5.0. $C_{15}H_{14}O_6$ requires C, 62.1; H, 4.9%). The crystals turn blue after 2 hr. at 110° *in vacuo* over P_2O_5 (Found: C, 62.5; H, 5.1%). The compound has a lower R_F (0.57) than (±)-robtin (0.76) in water-saturated butan-1-ol, but higher R_F values (0.49, 0.42, two spots, see below) than (±)-robtin (0.22) in 2% (v/v) acetic acid. This chromatographic behaviour is consistent with the conversion of a 4-oxo into a 4-hydroxyl group (cf. Roux, Maihs & Paulus, 1961*a*) during hydrogenation. (±)-3',4',5',7-Tetrahydroxyflavan-4-ol separates into its optical antipodes on paper chromatograms developed in water or 2% (v/v) acetic acid, as do the flavan-3,4-diols, flavan-3-ols, flavan-4-ols and flavanonols of the 'resorcinol series' (Roux, Maihs & Paulus, 1961*a*; compare Roberts & Wood, 1953).

On paper chromatograms the compound gives the following colours with the spray reagents indicated (cf. Roux & Maihs, 1960*b*): toluene-*p*-sulphonic acid (powder blue fading to lilac), ferric alum (blue), ammoniacal silver nitrate (black or brown) and bisdiazotized benzidine (deep yellow). When chromatograms were sprayed with ethanolic 2% (v/v) solutions of the following substituted quinonechlorimides, and then treated with ammonia vapour, the compound gave the colours: unsubstituted (yellow slowly developing blue edges), 2,6-dibromo- (yellow centre with powder-blue edges) and 2,6-dichloro- (yellow centre with deep-blue edges). The solid flavan-4-ol develops a deep red when exposed to sunlight, and compared with its analogues (Table 1) undergoes rapid self-condensation in the presence of dilute hydrochloric acid to form a red insoluble product, 'phlobaphene'.

(-)-Robtin (400 mg.) ($[\alpha]_D - 4.1^\circ$) from the heartwood of *R. pseudacacia* (Roux & Paulus, 1962) was hydrogenated in the presence of 200 mg. of platinum dioxide as described above, and gave 190 mg. of crystals of identical appearance and m.p. behaviour. Infrared-absorption spectra of these flavan-4-ols from synthetic and natural sources were superimposable over the range 2.5–15 μ . On paper chromatograms the product gave the same colour reactions and R_F values as those cited above. Paper chromatograms in 2% (v/v) acetic acid showed that the product was racemic.

(±)-3',4',5',7-Penta-acetylflavan. This compound, prepared by acetylation of synthetic (±)-tetrahydroxyflavan-4-ol (50 mg.), was crystallized from ethanol to give needles (44 mg.), m.p. 166–167° (Found: C, 60.0; H, 4.9; CO·CH₃, 42.5. $C_{25}H_{24}O_{11}$ requires C, 60.0; H, 4.8; CO·CH₃, 43.0%).

Table 1. Condensation and colour reactions of flavans related to 3',4',5',7-tetrahydroxyflavan-4-ol in the presence of hydrochloric acid

	Period before first* appearance of condensation product	Colour of condensation product	Colour of solution
(+)-3',4',5',7-Tetrahydroxyflavan-4-ol	15-30 sec.	Red-brown	Mauve→brown
(+)-3',4',5',7-Tetrahydroxyflavan-3,4-diol	3 min.	Light pink	Pink
(-)-3',4',5',7-Tetrahydroxyflavan-3-ol	5 min.	Buff	Colourless
(+)-3',4',5',7-Tetrahydroxyflavan-4-on-3-ol	No product	—	Pale yellow†
(-)-3',4',5',7-Tetrahydroxyflavan-4-one	‡	Brown	Deep orange§

* The compounds were treated as previously described (Roux & Paulus, 1961c).

† Colour due to conversion into robinetin.

‡ Brown precipitate on cooling.

§ Colour due to conversion into robtein.

They were identical with the penta-acetate of the tetrahydroxyflavan-4-ol derived from natural robbtin (m.p. 165-167°).

(±)-3',4',5',7-Tetramethoxyflavan-4-ol. The synthetic flavan-4-ol (300 mg.) was methylated with excess of diazomethane at -10° under conditions previously described (Roux & Paulus, 1960). The product settled from ethanol as oily globules that did not crystallize. After pouring into water the compound had m.p. 68-69° (Found: C, 65.2; H, 6.5; OMe, 36.8. $C_{19}H_{22}O_6$ requires C, 65.9; H, 6.4; OMe, 35.8%). When paper chromatograms were sprayed with toluene-*p*-sulphonic acid reagent and heated at 80°, the compound gave a brown-blue, fading to bright orange.

(±)-4-Acetoxy-3',4',5',7-tetramethoxyflavan. Acetylation of (±)-3',4',5',7-tetramethoxyflavan-4-ol (50 mg.) under above-mentioned conditions gave a product that did not crystallize from ethanol. When recovered from water, the product, m.p. 48-53°, settled slowly into a solid mass at room temperature (Found: OMe, 34.2; CO·CH₃, 10.7. $C_{21}H_{24}O_6$ requires OMe, 32.0; CO·CH₃, 11.1%).

(-)-Robbin and (+)-3',4',5',7-tetrahydroxyflavan-4-ol from (+)-dihydro-robinetin

Isolation of optically pure (+)-dihydro-robinetin. (+)-Dihydro-robinetin from *R. pseudacacia* has $[\alpha]_D +12^\circ$ to $+16^\circ$ (cf. Freudenberg & Hartmann, 1954; Roux & Paulus, 1962). Weinges (1958), however, cites $[\alpha]_D +29^\circ$ for the optically pure compound without giving the exact source or the method of resolution. The following procedure was found to be effective.

Natural dihydro-robinetin (500 mg.) in methanol (50 ml.) was streaked on 10 sheets of Whatman no. 3 (22½ in. × 18½ in.) chromatographic paper. The chromatograms were developed with 2% (v/v) acetic acid for 16 hr. by upward migration. The leading edge of the band was carefully marked under ultraviolet light; the forward-running ¾ in. wide portion of the band was cut and eluted with 70% (v/v) ethanol and the combined eluates gave large needles of (+)-dihydro-robinetin (110 mg.), m.p. 246°, $[\alpha]_D^{25} +27.8 \pm 0.4^\circ$ [c 1.0 in acetone-water (1:1, v/v)]. This sample gave an amorphous acetate, m.p. 82°, $[\alpha]_D^{25} +8.5 \pm 0.1^\circ$ in sym-tetrachloroethane (c 1.1). Under similar conditions the partly racemized (+)-dihydro-robinetin from *R. pseudacacia* crystallizes in small feathery needles, m.p. 223-226°; acetate, 113° [Freudenberg & Hartmann (1954) cite m.p. 143° for the acetate].

(-)-Robbin. Optically pure (+)-dihydro-robinetin (500 mg.) was dissolved in 5 ml. of methanol in a 25 ml. Erlenmeyer flask and the solution stirred with a strong magnetic stirrer. Dry A.R. zinc powder (1 g.), which had been deactivated previously by keeping in a damp condition for 10 days, was added to a solution of optically pure (+)-dihydro-robinetin (500 mg.) in methanol (5 ml.). The mixture was stirred mechanically and conc. hydrochloric acid (2.5 ml.) was added slowly over 1 hr., followed by another 1.75 ml. in the second hour. More deactivated zinc powder (total, 4-6 g.) was added in small quantities between each addition of hydrochloric acid as soon as a faint blue developed in the solution. Hydrochloric acid was added dropwise at a uniform rate over the periods indicated and the reaction was balanced throughout between a pale-blue and colourless or yellow solution by suitable addition of either zinc or hydrochloric acid. At no time was an excessively deep blue allowed to develop because this indicated that the reaction was proceeding too vigorously, or that the zinc dust was not sufficiently deactivated. Failure to observe a blue colour after 30 min. indicated that the conversion was not proceeding.

The acidic solution was filtered, diluted to 25 ml. with methanol and streaked on five sheets of Whatman no. 3 paper, 22½ in. × 18½ in.; the chromatograms were developed immediately in 2% (v/v) acetic acid. The slower-moving (R_F 0.25) of two bands was cut out and crystals (0.22 g.) separated readily from the concentrated eluate, m.p. 276° (decomp.) after sintering at 160-165°, $[\alpha]_D^{25} -56.8 \pm 0.5^\circ$ (c 0.9 in methanol). Mixed m.p. with synthetic and with natural robbtin from *R. pseudacacia* showed no depression. Unchanged (+)-dihydro-robinetin (0.12 g.) was recovered by cutting and eluting the second band, R_F 0.40.

Similar reduction of partly racemized (+)-dihydro-robinetin, $[\alpha]_D +14.3^\circ$ from *R. pseudacacia*, gave a partly racemized (-)-robbtin, $[\alpha]_D -24.9^\circ$, m.p. 276° (decomp.), which yielded an acetate, m.p. and mixed m.p. with synthetic and natural tetra-acetyl-(±)robbtin, 179-181°.

(+)-3',4',5',7-Tetrahydroxyflavan-4-ol. (-)-Robbin ($[\alpha]_D -56.8^\circ$) (700 mg.) in methanol (100 ml.) was hydrogenated in the presence of platinum dioxide (350 mg.) to yield, after recrystallization from water, 420 mg. of white crystalline needles, which did not melt below 345°, $[\alpha]_D^{25} +23.6 \pm 0.4^\circ$ [c 0.8 in acetone-water (1:1, v/v)] (Found: C, 62.3; H, 5.2. $C_{19}H_{24}O_6$ requires: C, 62.1; H, 4.9%). The infrared-absorption spectrum of the compound was almost identical with that of the racemate over the

Fig. 1. Comparison of infrared-absorption curves of (+)-3',4',5',7-tetrahydroxyflavan-4-ol and (+)-3',4',5',7-tetrahydroxyflavan-3,4-diol. —, (+)-3',4',5',7-Tetrahydroxyflavan-4-ol; ···, (+)-3',4',5',7-tetrahydroxyflavan-3,4-diol.

range 2.5–15 μ , and also very similar to that of (+)-3',4',5',7-tetrahydroxyflavan-3,4-diol (Fig. 1). The compound gave an amorphous acetate, m.p. 80°, $[\alpha]_D^{25} + 7.8 \pm 0.3^\circ$ (c 1.0 in *sym*-tetrachloroethane).

(-)-Butin [(+)-3',4',7-trihydroxyflavanone] and (+)-3',4',7-trihydroxyflavan-4-ol from (+)-fustin [(+)-3',4',7-trihydroxyflavanon-3-ol]

(-)-Butin. Optically pure (+)-fustin, $[\alpha]_D + 28.3^\circ$ (500 mg.), from *A. mearnsii* (Roux & Paulus, 1960) and from *A. pycnantha* (Roux, Maihs & Paulus, 1961b), was reduced with deactivated zinc under the conditions used for (+)-dihydorobinetin. (-)-Butin (130 mg.), m.p. 225–227°, $[\alpha]_D^{25} - 55.5 \pm 0.1^\circ$ (c 0.8 in methanol), resulted. Mixed m.p. with synthetic ($\pm$)-butin [m.p. 223–225° (Roux & Paulus, 1961a)] showed no depression.

By the same method ($\pm$)-fustin gave ($\pm$)-butin, m.p. 223–225°, which gave a methyl derivative, m.p. and mixed m.p. with synthetic trimethoxy-($\pm$)-butin (Roux & Paulus, 1961b) 117–118°.

(+)-3',4',7-Trihydroxyflavan-4-ol. Optically pure (-)-butin, $[\alpha]_D - 55^\circ$ (200 mg.), was hydrogenated as before (Roux & Paulus, 1961c) to give this flavan-4-ol, m.p. 94–96°, $[\alpha]_D^{25} + 24.2 \pm 0.2^\circ$ [c 1.0 in acetone–water (1:1, v/v)]. The compound formed a methoxy derivative, m.p. 137–138°, $[\alpha]_D^{25} - 4.4 \pm 0.2^\circ$ (c 0.8 in methanol). Mixed m.p. with ($\pm$)-3',4',7-trimethoxyflavan-4-ol [m.p. 137–138° (Roux & Paulus, 1961c)] showed no depression.

DISCUSSION

($\pm$)-3',4',5',7-Tetrahydroxyflavan-4-ol and ($\pm$)-3',4',7-trihydroxyflavan-4-ol, prepared by catalytic hydrogenation of the corresponding flavanones, both develop bright redness on exposure to sunlight, as do the corresponding flavan-3,4-diols

and the polymeric leuco-robinetinidin and leuco-fisetinidin tannins. The simultaneous presence of hydroxyl groups in the 4 and 7 positions and consequent ability to form *p*-methylene quinones (cf. Ivnäs & Lindberg, 1961) may be responsible for this phenomenon. These flavan-4-ols both show remarkable sensitivity towards self-condensation (Table 1), for which the reactive 4-hydroxyl group (cf. Roux & Paulus, 1961c) or a reactive *p*-methylene quinone group might be responsible.

Reduction of the flavanonols (+)-dihydorobinetin and (+)-fustin to the flavanone analogues (-)-robinetin and (-)-butin respectively is similar to the conversion by Pew (1948) of ($\pm$)-taxifolin [($\pm$)-3',4',5',7-tetrahydroxyflavanon-3-ol] into ($\pm$)-eriodictyol [($\pm$)-3',4',5',7-tetrahydroxyflavanone]. However, whereas reduction of 5-hydroxyflavanonols [e.g. taxifolin and ampeloptin (3',4',5',5',7-pentahydroxyflavanon-3-ol)] with hydrochloric acid–zinc dust in methanolic solution does not affect the 4-carbonyl group, presumably because of protection by the strong hydrogen bond, the 5-deoxyflavanonols [e.g. liquiritigenin (4',7-dihydroxyflavanone), robinetin and robinetin] are rapidly reduced to a deep blue. This may be ascribed to conversion into the corresponding flavan-4-ols, which develop a deep blue in the presence of mineral acids. Pew's reaction is, however, now shown to be possible with 5-deoxyflavanols when deactivated zinc and suitably modified conditions are used.

This selective reduction of the optically active flavanonols enables a prediction to be made of the absolute configuration of the corresponding flavanones. Thus (+)-dihydorobinetin (I, R = OH)

and (+)-fustin (I, R = H), which have a known (2*R*,3*R*)-configuration (Weinges, 1958; Roux & Paulus, 1960), are both converted into flavanones of (2*S*)-configuration. (-)-Robtin (II, R = OH) may therefore be designated as (2*S*)-3',4',5',7-tetrahydroxyflavan-4-one and (-)-butin (II, R = H) as (2*S*)-3',4',7-trihydroxyflavan-4-one. This agrees with the findings of Arakawa & Nakazaki (1960*a*, *b*), who showed that the natural laevorotatory flavanones, including a third member of the above series, (-)-liquiritigenin [(2*S*)-4',7-dihydroxyflavan-4-one], have the (2*S*)-configuration. The above conclusions are derived by independent methods, Arakawa & Nakazaki (1960*a*, *b*) using ozonization to L-malic acid, compared with interconversion from a compound of known absolute configuration in the present work.

The high negative rotations of the optically pure (-)-robtin ($[\alpha]_D -56.8^\circ$) and (-)-butin ($[\alpha]_D -55.6^\circ$) are of the same order as many natural flavanones (cf. Arakawa & Nakazaki, 1960*a*, *b*), and suggest that others of lower rotation probably occur in partly racemized form. Thus (-)-butin from black and golden wattles (Roux & Paulus, 1961*b*), $[\alpha]_D -18.5^\circ$, is partly racemized, and (-)-robtin from *R. pseudacacia*, $[\alpha]_D -4.2^\circ$ is almost completely racemized (compare above).

The reduction of (+)-flavanonol to (-)-flavanone supports previous concepts that flavonoid compounds arise from a common precursor, and suggests that the sequences (+)-fustin $\rightarrow$ (-)-butin $\rightarrow$ butein (2',3,4,4'-tetrahydroxychalcone) in the heartwoods of *A. mearnsii* and related species (cf. Roux & Paulus, 1960, 1961*a*, *b*; Roux, Maihs & Paulus, 1961*b*) and (+)-dihydorobinetin $\rightarrow$ (-)-

robtin $\rightarrow$ robtein in the heartwood of *R. pseudacacia* (Roux & Paulus, 1962) are feasible. Further, all these interconversions of flavonoid compounds may now be effected *in vitro* by simple hydrogenation or dehydrogenation of the heterocyclic ring, and it appears likely that such mechanisms may also operate *in vivo*. Dehydrogenation mechanisms may similarly be responsible for the formation of polymeric tannins from flavan-3,4-diols.

Catalytic hydrogenation in neutral solution of (-)-butin (II, R = H) to (+)-3',4',7-trihydroxyflavan-4-ol (III, R = H) is analogous to the conversion of (+)-fustin (2,3-*trans*) (I, R = H) into (+)-3',4',7-trihydroxyflavan-3,4-diol (2,3-*trans*-3,4-*cis*) (IV, R = H) under identical conditions (Roux & Paulus, 1960, 1961*c*). The assignment of a 3,4-*cis*-configuration to the flavan-3,4-diol is based on the high yield (65%) of isopropylidene derivative from the trimethoxy derivative (Clark-Lewis & Roux, 1959), evidence which appears to be valid in most but not in all instances (cf. Bokadia *et al.* 1961). The above analogy therefore suggests a 2,4-*trans*-configuration for (+)-3',4',7-trihydroxyflavan-4-ol (compare III, R = H, with IV, R = H). Identical considerations also apply to (+)-3',4',5',7-tetrahydroxyflavan-4-ol (III, R = OH), which has a similar infrared-absorption curve (Fig. 1) to the natural (+)-3',4',5',7-tetrahydroxyflavan-3,4-diol (IV, R = OH) [2,3-*trans*-3,4-*cis* (Roux & Paulus, 1962)] from *R. pseudacacia*. These compounds are formed by catalytic hydrogenation of (-)-robtin (II, R = OH) and (+)-dihydorobinetin (I, R = OH) (Weinges, 1958) respectively, the isopropylidene derivative of the (+)-3',4',5',7-tetramethoxyflavan-3,4-diol being formed in 60%

yield. A 2,4-*trans*-configuration was also assigned by Mitsui & Kasahara (1960) to the flavan-4-ol obtained by catalytic hydrogenation of flavanone in neutral solution.

The flavan-4-ols formed from (-)-butin (II, R = H) and (-)-robtin (II, R = OH) are therefore tentatively assigned the absolute configurations (2*S*,4*R*)-3',4',7-trihydroxyflavan-4-ol (III, R = H) and (2*S*,4*R*)-3',4',5',7-tetrahydroxyflavan-4-ol (III, R = OH) respectively.

SUMMARY

1. The analogues robtein, (+)-robtin and (+)-3',4',5',7-tetrahydroxyflavan-4-ol have been synthesized.

2. The sequence of conversions (+)-dihydro-robinetin → (-)-robtin → (+)-3',4',5',7-tetrahydroxyflavan-4-ol and (+)-fustin → (-)-butin → (+)-3',4',7-trihydroxyflavan-4-ol has been effected in each instance by means of a Clemmensen-type reduction followed by catalytic hydrogenation.

3. These interconversions establish the absolute configurations of flavanones as (2*S*), and those of the flavan-4-ols tentatively as (2*S*, 4*R*).

4. The biochemical significance of the ready conversion of flavanonol into flavanone is discussed.

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REFERENCES

- Arakawa, H. & Nakazaki, M. (1960*a*). *Liebigs Ann.* **663**, 111.
 Arakawa, H. & Nakazaki, M. (1960*b*). *Chem. & Ind.* p. 73.
 Bokadia, M. M., Brown, B. R., Kolker, P. L., Love, C. W., Newbould, J., Somerfield, G. A. & Wood, P. M. (1961). *J. chem. Soc.* p. 4663.
 Clark-Lewis, J. W. & Roux, D. G. (1959). *J. chem. Soc.* p. 1402.
 Freudenberg, K. & Hartmann, L. (1954). *Liebigs Ann.* **587**, 207.
 Freudenberg, K. & Hübner, H. H. (1952). *Chem. Ber.* **85**, 1181.
 Ivnäs, L. & Lindberg, B. (1961). *Acta chem. scand.* **15**, 1081.
 Mitsui, S. & Kasahara, A. (1960). *J. chem. Soc. Japan*, **81**, 1583.
 Pew, J. C. (1948). *J. Amer. chem. Soc.* **70**, 3031.
 Roberts, E. A. H. & Wood, J. D. (1953). *Biochem. J.* **53**, 332.
 Rosenmund, K. W. & Pfannkuch, E. (1922). *Ber. dtsh. chem. Ges.* **55**, 2357.
 Rosenmund, K. W. & Zetzsche, F. (1918). *Ber. dtsh. chem. Ges.* **51**, 594.
 Roux, D. G. & Maihs, A. E. (1960*a*). *Biochem. J.* **74**, 44.
 Roux, D. G. & Maihs, A. E. (1960*b*). *J. Chromat.* **4**, 65.
 Roux, D. G., Maihs, A. E. & Paulus, E. (1961*a*). *J. Chromat.* **5**, 9.
 Roux, D. G., Maihs, A. E. & Paulus, E. (1961*b*). *Biochem. J.* **78**, 834.
 Roux, D. G. & Paulus, E. (1960). *Biochem. J.* **77**, 315.
 Roux, D. G. & Paulus, E. (1961*a*). *Biochem. J.* **78**, 120.
 Roux, D. G. & Paulus, E. (1961*b*). *Biochem. J.* **80**, 62.
 Roux, D. G. & Paulus, E. (1961*c*). *Biochem. J.* **80**, 476.
 Roux, D. G. & Paulus, E. (1962). *Biochem. J.* **82**, 324.
 Schmidt, E. (1919). *Ber. dtsh. chem. Ges.* **52**, 409.
 Weinges, K. (1958). *Liebigs Ann.* **615**, 203.

Biochem. J. (1962) **84**, 421

The Influence of Potassium on Respiration and Glycolysis by Brain Slices

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Ashford & Dixon (1935) and Dickens & Greville (1935) observed that the respiratory and aerobic glycolytic activities of brain-cortex slices in a 'physiological saline' medium were greatly stimulated, and the anaerobic glycolytic activity inhibited, on addition of a high concentration (100 mM) of potassium chloride to the medium. These observations have been confirmed by various workers though the magnitude of the effects observed has varied [see, for example, Webb &

Elliott (1951)]. Except for recent studies by Cummins & McIlwain (1961), mentioned below, little information is available with regard to the influence of the low concentrations of potassium normally present in extracellular fluids or of moderate changes in these concentrations. In their detailed study on the effects of various cations on the metabolism of brain slices Dickens & Greville (1935) reported no results for the condition in which potassium alone was omitted from the medium. Gore & McIlwain (1952) reported that absence of potassium salts from the medium had no

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