

## Condensed Tannins

### 10. ISOLATION OF (-)-BUTIN AND BUTEIN FROM WATTLE HEARTWOODS

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The present study shows that in addition to the extractives described by Roux & Paulus (1960, 1961*a*) and Roux, Maihs & Paulus (1961) wattle heartwoods contain very low proportions of the isomeric compounds, (-)-butin [(*-*)-7:3':4'-trihydroxyflavanone] and butein (2:4-dihydroxyphenyl-3:4-dihydroxystyryl ketone).

#### EXPERIMENTAL AND RESULTS

All melting points were obtained in soda glass and are uncorrected. Mixed melting points are on molecular mixtures of compounds (Roux & Maihs, 1960). Analyses of C, H, methoxyl and acetyl are by Weiler and Strauss, Oxford. Infrared comparisons are by Dr J. R. Nunn, National Chemical Research Laboratory, C.S.I.R., Pretoria. Two-way chromatograms were developed in water-saturated butan-2-ol followed by 2% (v/v) acetic acid.

*Separation of (-)-butin from black-wattle heartwood.* The contents of tubes 146-160 from three 160-transfer Craig countercurrent partition separations, water-butan-2-ol-light petroleum (5:3:2) and an enriched flavonoid fraction being used for separations (Roux & Paulus, 1961*a*), were united. The organic phase was separated and the aqueous layer extracted with ethyl acetate. The combined organic phase and ethyl acetate extracts were reduced to 100 ml. and applied as a band (5 ml./sheet) to 20 sheets (22½ in. × 18½ in.) of Whatman no. 3 chromatographic paper. The chromatograms were developed by upward migration in 2% acetic acid. A reducing band  $R_f$  0.25 was located by ammoniacal silver nitrate on a test strip. Bands were cut and eluted with 70% ethanol. The eluents were evaporated to dryness under reduced pressure in a rotary evaporator, and the solids crystallized (charcoal) from water containing a little ethanol. Colourless flat plates (100 mg.) m.p. 223-224° were obtained from the methanol extract (360 g.) of the heartwood (9.9 kg.) (Roux & Paulus, 1961*a*) [Found: C, 58.1; H, 5.0; loss at 120° *in vacuo* (2 hr.), 11.4.  $C_{15}H_{12}O_6$ , 2H<sub>2</sub>O requires: C, 58.4; H, 5.2; H<sub>2</sub>O, 11.7%]. The anhydrous compound had m.p. 223-224°, not depressed by admixture with (±)-butin m.p. 223-224°;  $[\alpha]_D^{20}$  -18.6 ± 0.8° in methanol (*c*, 0.6) (Found: C, 66.4; H, 4.5.  $C_{15}H_{12}O_6$  requires: C, 66.2; H, 4.4%).  $\lambda_{max}$ . 234, 280 and 311 m $\mu$  in ethanol, and 254 and 336 m $\mu$  in a 0.1% soln. of sodium ethoxide in ethanol. The infrared-absorption spectrum of the compound (KBr disk) had strong bands at 3450 (OH), 1650 (pyrone C:O), 1600, 1530 and 1465 (aromatic C:C), 1280 (aromatic C:O) and 1120 (aryl-alkyl ether), but with low absorption over the range 1100-1000 cm.<sup>-1</sup> compared with the strong peak at 1015 cm.<sup>-1</sup> of fustin [secondary

aliphatic OH (?)]. The infrared-absorption and ultraviolet-absorption (cf. Harborne & Geissman, 1956) spectra and optical rotation suggested that the substance was (-)-butin. It gave resorcinol,  $\beta$ -resorcylic acid and protocatechuic acid on microfusion with KOH under temperature-controlled anhydrous conditions (Roux, 1958). On chromatograms the colours given with spray reagents were yellow (bis-diazotized benzidine), green-grey (ferric alum) and yellow (under ultraviolet light after treatment with ammonia).

*O-Trimethyl-(-)-butin.* (-)-Butin (70 mg.) in methanol (20 ml.) was methylated at -10° for 12 hr. with excess of dry ethereal diazomethane. *O*-Trimethyl-(-)-butin (40 mg.) was recrystallized from ethanol as colourless plates, m.p. 114-115°;  $[\alpha]_D^{20}$  -16.3 ± 0.2° in tetrachloroethane (*c*, 0.9) (Found: C, 68.9; H, 5.8; OMe, 30.3.  $C_{18}H_{18}O_6$  requires: C, 68.8; H, 5.8; OMe, 29.6%).

*(-)-Butin from golden-wattle heartwood.* (-)-Butin (100 mg.) was isolated from 250 g. of methanol extract of golden-wattle heartwood (5.1 kg.) as described above (cf. Roux *et al.* 1961); m.p. 223-224°;  $[\alpha]_D^{20}$  -18.7 ± 0.8° in methanol (*c*, 0.5) and -10.1 ± 0.8° in acetone-water (1:1, v/v) (*c*, 0.7). The ultraviolet- and infrared-absorption curves were the same as those of (-)-butin from black wattle, and a mixed m.p. of these compounds showed no depression.

*Triacetyl-(-)-butin.* (-)-Butin (70 mg.) from golden-wattle heartwood was acetylated with 0.3 ml. of pyridine and 0.35 ml. of acetic anhydride at room temperature for 2 hr. The product obtained by pouring into water crystallized from ethanol in white clusters (37 mg.) and had m.p. 121-122°. Recrystallization gave white shining platelets m.p. 121-122°;  $[\alpha]_D^{20}$  -12.5 ± 0.3° in tetrachloroethane (*c*, 0.5) (Found: C, 63.3; H, 4.6.  $C_{21}H_{18}O_8$  requires C, 63.2; H, 4.7%).

The presence of butin in the heartwoods of green (*Acacia decurrens*) and silver (*Acacia dealbata*) wattles was shown by paper chromatography.

*Butin from commercial quebracho extract.* Contents of tubes 145-160 of a Craig separation of a flavonoid-enriched fraction (320 g.) of commercial quebracho extract (Roux & Paulus, 1961*b*) were combined, and the components separated on paper sheets with 2% (v/v) acetic acid as above. A band corresponding to butin was cut and eluted but failed to crystallize under identical conditions. Paper chromatograms showed that the fraction was complex but that butin was the predominant component. Running the fraction again on two sheets of Whatman no. 3 paper in 2% acetic acid gave 1.5 mg. of plates from ethanol-water, m.p. 220-221° not depressed by admixture with (±)-butin, m.p. 220-222°.  $\lambda_{max}$ . 234, 280 and 310 m $\mu$  in ethanol, and

254 and 336 m $\mu$  in a 0.1% soln. of sodium ethoxide in ethanol. Colour reactions on paper chromatograms were identical with those of (-)-butin from black-wattle wood.

**Synthesis of ( $\pm$ )-butin.** Alkaline isomerization of butein (Saiyad, Nadkarni & Wheeler, 1937) gave a 32% yield of ( $\pm$ )-butin, m.p. 223–224°, whereas acid isomerization (Geissman & Clinton, 1946) gave 7% of ( $\pm$ )-butin, m.p. 225–226°. Reichel, Burkart & Muller (1942) report a 37% yield of ( $\pm$ )-butin from butein by acid isomerization. ( $\pm$ )-Butin was separated from unchanged butein by chromatography on Whatman no. 3 paper with 2% acetic acid. Ultraviolet-absorption (range 230–340 m $\mu$ ) and infrared-absorption (range 2.5–15  $\mu$ ) curves of synthetic and natural butins were superimposable. Their  $R_f$  values were identical in 2% acetic acid (0.22) and in water-saturated butan-2-ol (0.92), with (+)-fustin (0.37, 0.82 respectively) as a reference substance.

**O-Trimethyl-( $\pm$ )-butin.** ( $\pm$ )-Butin was methylated as was (-)-butin and gave a product with m.p. 118–119°. The mixed m.p. with O-trimethyl-(-)-butin (m.p. 114–115°) was m.p. 118°. The infrared-absorption curves of these substances were identical over the range 2.5–15 m $\mu$ .

**Triacetyl-( $\pm$ )-butin.** ( $\pm$ )-Butin was acetylated as was (-)-butin. The product had m.p. 124–126°. The mixed m.p. with triacetyl-(-)-butin (m.p. 121–122°) was 123–125°.

**Isolation of butein from golden-wattle heartwood.** Those components in tubes 146–160 from the Craig counter-current separation which remain at the origin during subsequent separation of (-)-butin on paper sheets, were cut out and eluted with 90% ethanol. Fisetin and a yellow substance were shown present in the eluents by paper chromatography with butan-1-ol-acetic acid-water (6:1:2, by vol.). The solids (2.5 g.) were applied to Whatman no. 3 sheets, which had been washed previously with water for 24 hr., and the chromatograms developed in the above-named solvent system. A yellow band running immediately in front of fisetin was cut and eluted with 70% ethanol. The yellow solids were recrystallized from ethanol containing a little water to yield bright-yellow crystals, m.p. 211° (8 mg. from 5.1 kg. of heartwood). The infrared-absorption curves of the substance and of synthetic butein (m.p. 212°) were superimposable. A mixed m.p. of these substances showed no depression (211°).

Butein was also found in black-wattle (*Acacia mearnsii*), silver-wattle (*A. dealbata*) and green-wattle (*A. decurrens*) heartwood extracts.

## DISCUSSION

(-)-Butin and the corresponding chalkone, butein, have been shown to occur as minor components in the heartwood extracts of *Acacia mearnsii* and *A. pycnantha* by isolation and direct comparison with synthetic compounds. These

compounds add to the already complex pattern of inter-related heartwood components, all of which are based on a combination of resorcinol and catechol nuclei (cf. Roux & Paulus, 1960, 1961a).

Optically active butin has hitherto not been isolated from natural sources, and the absolute configuration of natural and synthetic butins has not been established. (-)-Butin [( $\pm$ )-7:3':4'-trihydroxyflavanone] from wattle heartwoods ( $[\alpha]_D^{25}$  -18.7°) is likely to have the same (2S) configuration as (-)-liquiritigenin [( $\pm$ )-7:4'-dihydroxyflavanone] from *Glycyrrhiza glabra* ( $[\alpha]_D^{25}$  -36°) (Arakawa & Nakazaki, 1960a, b). (-)-Butin is therefore likely to have the same configuration at the asymmetric carbon atom 2 as the associated and related compounds (+)-fustin, (-)-fisetinidol and (+)-7:3':4'-trihydroxyflavan-3:4-diol present in wattle heartwoods (Roux & Paulus, 1960, 1961a; Weinges, 1958, 1959).

## SUMMARY

1. (-)-Butin and butein have been isolated from black- and golden-wattle heartwoods.
2. Traces of butin exist in quebracho heartwoods.

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