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Quebrachitol Synthesis in Hevea brasiliensis

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Gas-liquid chromatography was used in conjunction with a trapp system for the recovery of effluent $^{14}CO_2$ to estimate both the absolute concentration and the specific activity of each constituent in mixture of ^{14}C-inositols.

Detached Hevea *leaflets were shown to convert myo-inositol to L-inositol, and L-inositol to quebrachitol. Both reactions were apparently several hundred times faster than those of an alternative route mediated by D-bornesitol, and it is concluded that in* Hevea *quebrachitol is synthesised almost exclusively by the L-inositol pathway.*

The experimental data were used to assess the rate at which quebrachitol might be formed in leaf latex, and an essentially similar value was calculated from published information for trunk latex. The implied turnover was relatively slow, suggesting that although quebrachitol is the chief water-soluble constituent of the latex, its synthesis is a quantitatively minor aspects of latex metabolism.

Knowledge of the plant-taxonomic distribution of different inositols comes largely from a series of about forty publications by Plouvier[1]. Although the survey is still incomplete, it is well recognised that some plants and plant families are characterised by the accumulation of particular inositols; quebrachitol (2-0-methyl-L-inositol) itself accumulates to a marked degree in a small number of genera representing eight, for the most part distantly-related dicotyledonous orders (Asterales, Apocynales, Euphorbiales, Proteales, Rhamnales, Stantalales, Sapindales, and Urticales). This coincidentally includes all the more important sources of natural rubber[2], and at a concentration of about 1% weight/volume quebrachitol is in fact the main non-rubber constituent of *Hevea* latex. Apart from biochemical interest in what might be thought a major metabolic function, this raises the prospect that quebrachitol is, in *Hevea*, a major factor determining the turgor status of the latex vessels, and hence the outflow of latex when the tree is tapped.

The quebrachitol content of the latex is scarcely affected by moderate-intensity tapping[3], so that the amounts drained from the tree are presumably replenished by an increase in the rate of synthesis. A tapping-dependant acceleration of quebrachitol formation could thus be responsible for restoring the turgidity of the latex vessels, and indeed account for the amenability of the trees to repetitive tapping.

Taking the view that all the other inositols are formed ultimately from *myo*-inositol[4], the synthesis of quebrachitol would be expected to involve either epimerisation to L-inositol and subsequent methylation, or, alternatively, the appearance of bornesitol (1-0-methyl-*myo*-inositol) as the intermediate *(Figure 1)*. Work with other plants has shown that both routes may be functional.

In leaf-feeding experiments with *Artemisia vulgaris* (Compositae; Asterales), Scholda *et al.*[4] obtained a 13% conversion of *myo*-inositol to quebrachitol, and

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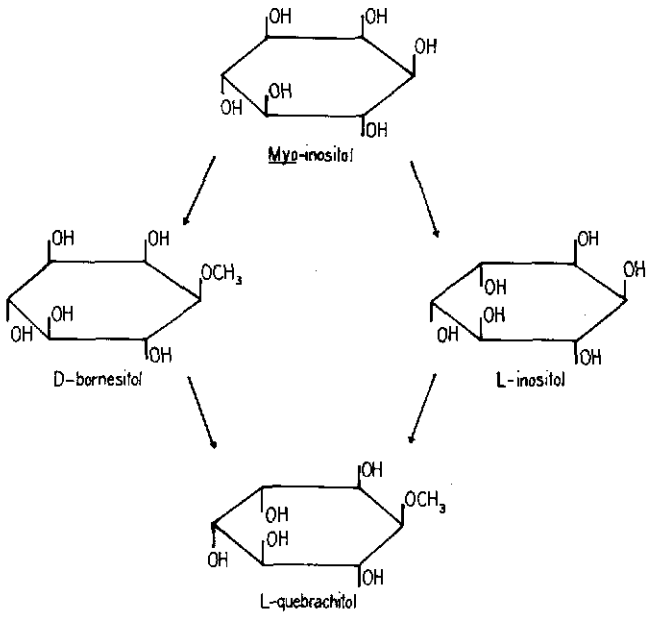

Figure 1. Alternative metabolic pathways from myo-*inositol to quebrachitol.*

a 12% conversion when L-inositol was the precursor. Although the conversion of *myo*-inositol to L-inositol was surprisingly low, the absence of bornesitol supported the conclusion that the L-inositol pathway was operative. This was confirmed[5] by evidence that after photosynthesis in $^{14}CO_2$, the appearance of ^{14}C-*myo*-inositol was followed by slight labelling of L-inositol, and a progressive increase in ^{14}C-quebrachitol. Bornesitol was again not detected, nor, in leaf-feeding experiments, was ^{14}C-bornesitol epimerised to quebrachitol.

In contrast to the position in *Artemisia*, experiments with *Acer pseudoplatanus* (Aceraceae; Sapindales) showed that photosynthesis in $^{14}CO_2$ this time yielded radioactive bornesitol, together with ^{14}C-*myo*-inositol and ^{14}C-quebrachitol; but no ^{14}C-L-inositol. In the course of a further five days in $^{12}CO_2$ the ^{14}C-bornesitol disappeared, and there was an apparently conpensating increase in ^{14}C-quebrachitol[5,6]. Radioactive L-inositol also failed to appear in leaves fed ^{14}C-*myo*-inositol, although there was substantial conversion to radioactive bornesitol and quebrachitol. ^{14}C-Quebrachitol was indeed produced in leaves fed ^{14}C-L-inositol, but the conversion was about 5% of the tracer in two days compared with an 80% conversion of bornesitol under the same conditions. Translation of these results into absolute terms is not possible in the absence of data for the specific activities of the tracers, but the failure to convert *myo*-inositol to L-inositol clearly indicates that in this species it is the bornesitol pathway which is operative.

It was the object of the present work to discover which of the two routes is

responsible for the formation of quebrachitol in *Hevea brasiliensis*.

EXPERIMENTAL

Inositols

L-Quebrachitol was provided by Professor G.F.J. Moir from material isolated from *Hevea* latex at the RRIM Experiment Station, Sungei Buloh, and was purified by Soxhlet-extration into ethanol. Crystallisation from the boiling extract and two recrystallisations, by adding acetone to concentrated solutions in 50% aqueous ethanol, yielded quebrachitol, melting point 189°C. Part of the quebrachitol was demethylated by boiling with 65% weight/volume hydriodic acid for 30 min under reflux, and the product was crystallised from ethanol: water (approx. 8:1 volume/volume) to give L-inositol, melting point 245.5°C. D-(−)-Bornesitol (1-0-methyl-*myo*-inositol) was not otherwise obtainable, and in view of its known occurrence in *Rhamnus* spp.[7] was isolated from leaves of *R. catharticus* collected at Wicken Fen, Cambridgeshire, England.

A boiling-water extract (3.5 litres) of 1 kg dry leaf powder was treated with 10% weight/volume basic lead acetate until there was no further precipitate, and the filtrate, rendered lead-free with H_2S, was passed through a 20 x 3.6 cm charcoal: Celite 535 column (1:1 weight/weight) to remove sucrose and other oligosaccharides. The solution was concentrated to 800 ml in a rotary evaporator, made strongly alkaline with 100 ml 2 *M* NaOH, filtered, and boiled for 1 h under reflux to oxidise monosaccharides to sugar acids before being neutralised and passed through columns (30 x 3.6 cm) of Dowex 1-acetate$^-$ and Dowex 50-H^+ ion-exchange resins.

The effluent, containing 48 g solids, was mixed with 80 g Celite 535, oven-dried at 90°C, and Soxhlet-extracted with acetone for 136 h to yield 12 g acetone-soluble material. Portions (2–3 g) of the latter were dissolved in 50% volume/volume aqueous acetone, transferred to a 50 x 2.7 cm column of Whatman CC 31 cellulose powder, and eluted with acetone: water (17:3 volume/volume *Solvent A)*. Fractions from the column were monitored by paper chromatography (butan-1-ol: pyridine:water:acetic acid, 60:40:30:3 *volume/volume/volume/volume: Solvent B)*, the inositols being detected with alkaline silver nitrate[8]. Bornesitol, identified by its location between L-inositol and quebrachitol markers, emerged from the column after 1.2 litres of solvent had passed through, and was further purified by chromatography on Whatman 3 MM paper *(Solvent B)*, and elution from the appropriate part of the paper with water. The products from successive column and paper separations were mixed with Celite 535, oven-dried, and Soxhlet-extracted into ethanol; the identity of bornesitol (0.5 g; melting point 205°C) crystallising on the walls of the flask was confirmed by its IR spectrum and, following acetylation, a gas-chromatographic retention time indistinguishable from that for the acetate from authentic ^{14}C-bornesitol.

U-^{14}C-D-Bornesitol and U-^{14}C-L-quebrachitol, isolated respectively from leaves of *Myosotis arvensis* and *Acer pseudoplatanus* after photosynthesis in $^{14}CO_2$, were a gift from Dr N. Schilling. Additional ^{14}C-quebrachitol was isolated from *Acer* seedlings after fifteen days in $^{14}CO_2$, by paper-chromatographic fractionation of a charcoal-treated and deionised hot-water extract. Notwithstanding separation in four solvents

(phenol:water:acetic acid:M Na_2EDTA, 400:134:5:5, weight/volume/volume/volume; benzene:dimethylformamide: water, 100:40:1, volume/volume/volume; and *Solvents A* and *B*), gas-liquid chromatographic (GLC) analysis of a sample pretreated for one hour at 20°C with sodium borohydride (0.6% weight/volume in *M* NaOH: excess reagent decomposed by acidification) revealed a small but highly-radioactive sorbitol peak, indicative of contamination by glucose. This was eliminated by incubation with 0.25% weight/volume glucose oxidase (Sigma Chemical Company, Type IX) in 0.1 *M* acetate buffer, pH 4.7 for 36 h at ethanol:2 *M* formic acid mixture (4:1 gluconic acid and buffer by passage through miniature Dowex 1-acetate$^-$ and Dowex 50-H^+ columns.

U-^{14}C-L-inositol was obtained by demethylation of ^{14}C-quebrachitol in boiling hydriodic acid, and separation of the L-inositol (detected by autoradiography) on paper chromatograms developed in *Solvent A*. U-^{14}C-*myo*-Inositol was purchased from the Radiochemical Centre, Amersham, England.

Plant Material and Incubation Technique

Leaves and latex were obtained from seedlings of *Hevea brasiliensis* growing in the greenhouse at Brickendonbury. In the case of latex experiments, about 0.25 g latex was collected directly into specimen tubes containing ^{14}C-tracer which had previously been evaporated to dryness in a nitrogen stream. The tubes were stoppered, and agitated intermittently during incubation for 5 h at 21°C. The rubber was then coagulated by adding 2 ml of an ethanol:2 *M* formic acid mixture (4:1 volume/volume), and the supernatant and aqueous washings concentrated for chromatography in *Solvent A (see below, under* Extraction and Purification of Leaflet Inositols).

Preliminary leaf analyses indicated that the quebrachitol content increased rapidly as the initially pendant leaflets assumed a horizontal orientation, and all the leaflet experiments were based on leaves at this stage of development. The leaves for each experiment were taken from single vigorously-growing shoots, and were trimmed by cutting off the side leaflets and the distal half of the centre leaflet, to leave a single abbreviated lamina at the end of the petiole.

A 1 cm zone in the middle of each petiole was scraped free of green laticiferous tissue, and the exposed xylem cylinder was cut through under water. The leaflet was then quickly transferred to a narrow tube containing 0.1–0.2 ml of a solution of the radioactive tracer. Uptake of the solution and subsequent water rinses were hastened by placing the leaflets in an air current, after which they were taken to the greenhouse and kept in covered glass tanks provided with water and a slow through-flow of moist air to the outside of the greenhouse.

Extraction and Purification of Inositols from Individual Leaflets

Four to seven days after feeding, the petioles and attached laminae (subsequently referred to as half-leaflets) were shredded into 10 ml of 4:1 volume/volume ethanol:water mixture, and extracted by boiling for 1 h under reflux. The extraction was repeated with a further 10 ml of aqueous ethanol, and the combined extracts were evaporated to dryness in a nitrogen stream at 45°C. The residues were dissolved in 2 ml of 0.5 *M* NaOH and heated for 1 h in a boiling

water bath (to oxidise sugars to sugar acids) before being neutralised with acetic acid, and passed through columns (60 x 8 mm) of Dowex 1-acetate$^-$ and Dowex 50-H$^+$.

The inositols in the effluent solution were subsequently acetylated to provide volatile products suitable for analysis by GLC. It was however found that direct analysis of inositol mixtures was subject to large errors due to co-chromatography of the acetates with each other, a tendency clearly demonstrated by analysis of test mixtures in which only one component was radioactive. The difficulty was largely avoided by prior separation of the inositols on one-dimensional paper chromatograms *(Solvent A)*, and elution of individual *myo*-inositol, L-inositol/bornesitol and quebrachitol fractions (located by autoradiography and reference to marker spots) from the paper. When this technique was applied to a mixture containing 1 mg each of L-inositol, quebrachitol, and ^{14}C-*myo*-inositol, GLC separation of the final acetylated fractions yielded L-inositol and quebrachitol peaks respectively accounting for only 0.5% and 0.07% of the total radioactivity.

Acetylation

The method was adapted from that described by Jones and Albersheim[9] for the GLC analysis of mixtures of alditols, using arabitol as the internal standard instead of *myo*-inositol. The eluates from the paper chromatograms, usually containing 0.5–2 mg inositol, were collected in 15 ml glass ampoules, supplemented with 0.1 ml 1% weight/volume arabitol and 0.5 ml 0.15 *M* sodium acetate (acetylation catalyst), evaporated in a nitrogen stream at 45°C, and dried *in vacuo* over phosphorus pentoxide overnight. Acetic anhydride (0.5 ml) was added, and the inositols and arabitol were acetylated by heating the sealed ampoules for 3 h at 120°C. The solutions were evaporated in a nitrogen stream at room temperature, and the reaction products were dissolved in 1 ml of redistilled chloroform ready for GLC.

Gas-liquid Chromatography

The instrument was a Perkin-Elmer Model F 30 Gas Chromatograph equipped with flame-inosisation detectors, and was operated isothermally at 210°C. No single liquid phase was found which would resolve bornesitol from both L-inositol and quebrachitol, but good separation was achieved on a 3.7 m x 6 cm outer diameter stainless steel column packed with a mixture of OV 17 (1% weight/weight) and SP 1000 (0.5% weight/weight) on Chromosorb G 60-80.

Quantitative Analysis

The recorder-trace peaks corresponding to the individual inositols and the arabitol standard were cut from photocopies of replicate GLC separations, and weighed. The absolute concentration of each inositol was estimated by reference to standard mixtures, containing 1 mg of each component, which had been chromatographed, eluted, and acetylated at the same time as the experimental samples.

The ^{14}C-content of each inositol was estimated by trapping the $^{14}CO_2$ emerging from the GLC detector head. An eight-way stainless steel/Teflon tap manufactured in the Malaysian Rubber Producers' Research Association Workshop allowed effluent gases to be led from the detector through 0.3 cm inner diameter stainless steel tubing (heated electrically, as was the tap itself, to avoid

premature condensation of water) into paired gas-washing tubes attached to exit ports around the periphery of the tap. Each tube contained 4 ml of 2-methoxyethanol: redistilled ethanolamine (4:1 volume/volume) and 4 ml of phosphor solution (0.6% weight/volume butyl PBD in toluene): the gas flow was operated under slight suction to maintain a layer of foam above the liquid, and, at the same time, to dispense with the need for gas-tight connections to the detector head.

Experiments showed that if three gas-washing tubes were connected in series, 96% of the recovered radioactivity was already trapped in the first tube, and 3.9% in the second. In common with experience elsewhere[10,11], however, the overall recovery of radioactivity injected into the column did not usually exceed 60%–70%, and the results were expressed as percentages of the radioactivity actually recovered, without attempting to adjust for a hypothetical 100% yield.

RESULTS

Inositol Content of Young Hevea *Leaflets*

Analyses of ten superficially similar leaflets from different plants revealed very divergent contents of 0.3–2.2 mg quebrachitol, 0.4–2.4 ml L-inositol and 0.2–1.6 mg *myo*-inositol. Although the scatter of results was partly attributable to a general increase in inositol content as the leaves matured, there were also major plant-to-plant differences, with all the leaves from some individuals being characterised by abnormal levels of one or other constituent. Bornesitol was detected in small amount, rarely more than 0.1 ml per half-leaflet, in about one quarter of the plants examined. A representative GLC analysis is shown in *Figure 2*.

The component *Y* appeared in all the inositol extracts as a peak in the pentitol region. It was, however, readily separable from arabitol, xylitol and ribitol, and since no peak was obtained if the sample was not acetylated, was tentatively regarded as a modified inositol. In ^{14}C-feeding experiments, *Y* showed no uptake of radioactivity beyond the low background level also acquired by the arabitol peak, and further characterisation was not attempted.

Feeding Experiments with ^{14}*C*-myo-*inositol*

In a typical experiment, analysis of the inositol fractions obtained from a half-leaflet fed four days previously with ^{14}C-*myo*-inositol showed a 13% conversion of the tracer to L-inositol, a 4% yield of ^{14}C-quebrachitol, and less than a 0.2% conversion to bornesitol. Analysis of the boiled zero-time control confirmed that there was only slight co-chromatography of the tracer with the other inositols, about 0.5% of the recovered radioactivity being associated with L-inositol/bornesitol, and 0.1% with quebrachitol.

The conversion of *myo*-inositol to L-inositol was also followed in a time-course experiment, in which ten half-leaflets were each fed 0.3 μg (9.6×10^5 disintegrations per minute) ^{14}C-*myo*-inositol. A hollow steel punch 13 mm in diameter was used to cut one disc from each lamina on each of the first four days, and again on the seventh day, by which time the leaflets were turning yellow. Each set of discs was extracted with boiling aqueous ethanol, and a further control sample, cut from unfed leaflets, was similarly extracted with aqueous ethanol containing 5.7×10^5 dpm of the same tracer. Unlabelled bornesitol (0.5 mg) was added to each extract

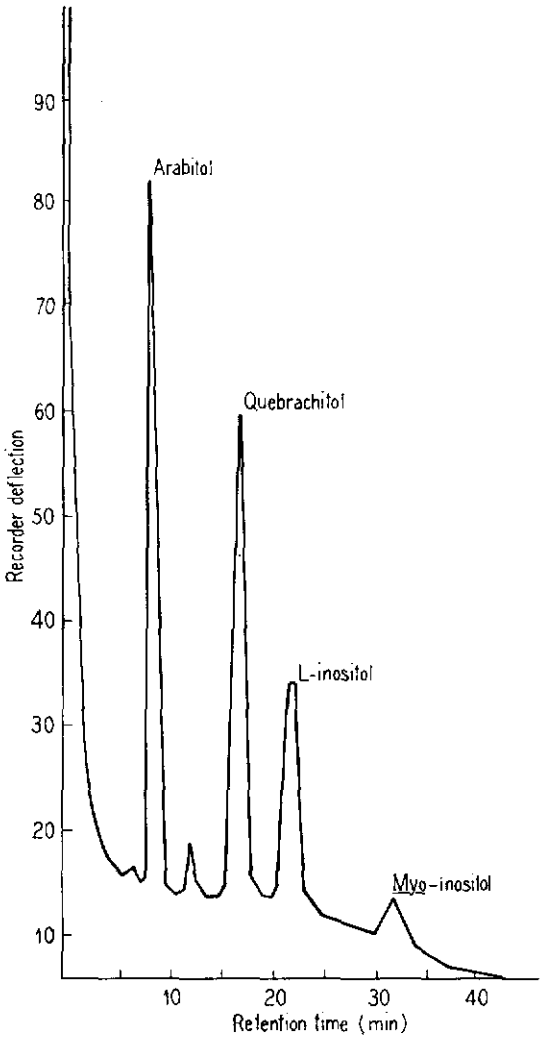

Figure 2. Typical gas-chromatographic separation of acetylated inositols from a leaflet extract, augmented with arabitol as internal standard. Bornesitol, when present, appeared as a peak between quebrachitol and L-inositol.

as carrier for possible traces of ^{14}C-bornesitol, and the inositol fractions were separated and analysed as described in the Experimental section.

The results *(Figure 3)* showed a rapid initial conversion to ^{14}C-L-inositol, with the rate decreasing as the leaflets aged, and the amount of ethanol-soluble radioactivity diminished. The formation of radioactive L-inositol was followed by a slow increase in ^{14}C-quebrachitol, whereas the ^{14}C-content of the bornesitol remained unchanged at a near-background level, possibly denoting the extent of cross-contamination from the other inositols.

These results were consistent with the synthetic pathway mediated by L-inositol *(Figure 1)*, but clearly did not exclude the possibility that the low content of bornesitol, labelled or otherwise, was itself indicative of a rapid conversion of bornesitol to quebrachitol. If this were the case, the addition of unlabelled bornesitol to the feeding solution would be expected to depress the formation of ^{14}C-quebrachitol, whereas conversely the L-inositol pathway should be open to competition from unlabelled L-inositol.

In each of two further experiments, three half-leaflets were accordingly fed ^{14}C-*myo*-inositol (0.06 μg, 2 x 10^5 dpm) by itself, or admixed with 1 mg or 1.5 mg L-inositol or bornesitol. The leaflets were extracted four days after feeding, and zero-time control extracts were made at the same time by plunging other half-leaflets into boiling ethanolic solutions of the appropriate tracer mixtures. In both experiments, large differences in the extent to which the *myo*-inositol was utilised made direct comparison of the results difficult, but a remarkably consistent labelling pattern emerged when the data were instead expressed as percentages of the combined ^{14}C-content of the L-inositol, bornesitol and quebrachitol fractions *(Table 1)*. About 80% of the total was invariably represented

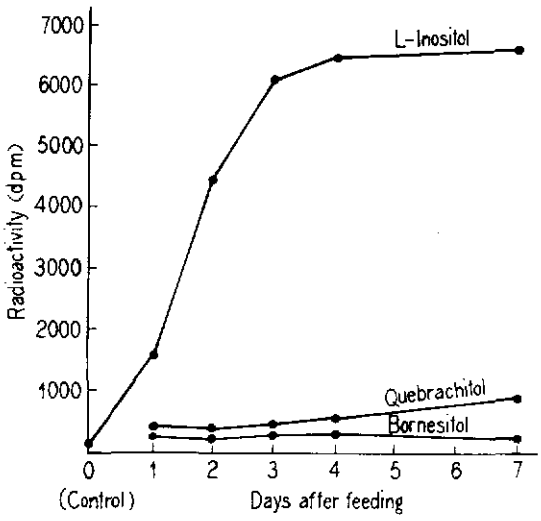

Figure 3. Time-curve of the conversion of ^{14}C-myo-inositol to ^{14}C-L-inositol and ^{14}C-quebrachitol in discs cut from detached leaflets.

by L-inositol, with ^{14}C-quebrachitol accounting for most of the remainder.

Gas-liquid chromatographic analyses showed that the 1 mg or 1.5 mg bornesitol supplements were at least fifty times greater than the amount conceivably present in the leaflets, and which, although not detected as a distinct component, might still have been masked by the adjacent L-inositol peak. Assuming complete equilibration of the added bornesitol with any existing pools, a bornesitol-mediated pathway could thus have been subject to a major dilution effect, with virtual suppression of the formation of ^{14}C-quebrachitol, and a compensating increase in ^{14}C-bornesitol. In fact the results showed no significant change in the yield of radioactive quebrachitol.

The L-inositol supplements represented by contrast only a 1.5- or 2-fold increase over the substantial amounts already in the leaflets, and might have given, at best, only a 33% or 50% reduction in ^{14}C-quebrachitol. An effect of this magnitude was not achieved, but both sets of results did show a limited shift in the labelling pattern, in one case by as much as 18%, away from quebrachitol.

Feeding Experiments with ^{14}C-L-inositol and ^{14}C-bornesitol

Although the above experiments established the ability of detached leaflets to convert *myo*-inositol to L-inositol, it was still not entirely certain that it was the L-inositol which gave rise to quebrachitol.

Evidence for this was sought by feeding half-leaflets with ^{14}C-L-inositol (0.2 mg, 6.7×10^4 dpm) or ^{14}C-bornesitol (1 mg, 1.6×10^5 dpm) as starting materials. The greater weight of bornesitol was deliberate, to compensate for the high L-inositol content of the leaflets, and to ensure that any metabolism of bornesitol might be deemed to take place under substrate-saturation conditions. The leaflets were extracted four days after feeding, and the usual zero-time control extracts were made by plunging two additional half-leaflets into boiling 80% volume/volume ethanol containing further aliquots of the two tracers.

Analysis of the separated quebrachitol fractions showed that there had been a 6% conversion of the ^{14}C-L-inositol, as opposed to a doubtfully significant 0.3% conversion of bornesitol *(Table 2)*. The leaflets used in this experiment contained exceptionally large amounts of L-inositol (3.7–4.1 mg), so that in absolute terms the conversion of L-inositol to quebrachitol was possibly three hundred times greater than the utilisation of bornesitol.

TABLE 1. CONVERSION OF ^{14}C-*MYO*-INOSITOL TO L-INOSITOL, QUEBRACHITOL AND BORNESITOL IN DETACHED LEAFLETS

Experiment	Feeding solution	Distribution of radioactivity (%) L-inositol	Quebrachitol	Bornesitol
1	^{14}C-*myo*-inositol (0.06 μg, 2 x 10^5 dpm)	77.0	22.6	0.4
	+ 1.5 mg bornesitol	75.6	23.4	1.0
	+ 1.5 mg L-inositol	78.1	21.5	0.4
2	^{14}C-*myo*-inositol (0.06 μg, 2 x 10^5 dpm)	84.3	15.2	0.5
	+ 1 mg bornesitol	83.7	14.6	1.7
	+ 1 mg L-inositol	86.7	12.4	0.9

TABLE 2. CONVERSION OF ^{14}C-L-INOSITOL AND ^{14}C-BORNESITOL TO ^{14}C-QUEBRACHITOL IN DETACHED LEAFLETS

Tracer	Time from feeding	^{14}C-content of quebrachitol peak (% of recovered ^{14}C-activity)	Implied percentage conversion to quebrachitol	Absolute conversion[a] (nmoles/half-leaflet/4 days)
^{14}C-L-inositol (0.2 mg, 6.7 x 10^4 dpm)	0 (control)	0.65	–	–
	4 days	7.16	6.5	2 400
^{14}C-bornesitol (1 mg, 1.6 x 10^5 dpm)	0 (control)	0.21	–	–
	4 days	0.55	0.34	8

[a]Calculated from the percentage yield of ^{14}C-quebrachitol, and the specific activities of ^{14}C-L-inositol and ^{14}C-bornesitol recovered at the end of the experiment.

In a second experiment there was a 4% conversion of L-inositol, while the turnover of bornesitol, less than 0.2%, was too low to be considered significantly greater than the boiled control.

The formation of quebrachitol was also measured in an experiment in which two half-leaflets were extracted seven days after feeding with ^{14}C-L-inositol (0.7 mg, 2.8 x 10^5 dpm) or ^{14}C-*myo*-inositol (1 mg, 2.8 x 10^5 dpm). Whereas the ^{14}C-content of the separated inositol fractions accounted for all (101%) of the initial L-inositol radioactivity, there was only a 38% recovery of the *myo*-inositol activity.

TABLE 3. CONVERSION OF ^{14}C-L-INOSITOL AND ^{14}C-*MYO*-INOSITOL TO ^{14}C-QUEBRACHITOL IN DETACHED LEAFLETS

Tracer	Percentage of recovered radioactivity			Absolute conversion (nmoles/7 days) to		
	L-inositol	*myo*-inositol	quebrachitol	L-inositol	*myo*-inositol	Quebrachitol
^{14}C-L-inositol (0.7 mg, 2.9 x 10^5 dpm)	72.8	0.6	26.6	–	14	5 700
^{14}C-*myo*-inositol (1 mg, 2.8 x 10^5 dpm)	6.0	31.9	0.1	620	–	14

Leaflets extracted seven days after feeding. Results corrected by subtraction of zero-time control values, and absolute conversion calculated from the specific activities of residual tracer: the latter represented 86% (L-inositol) and 77% (*myo*-inositol) of the specific activity of the tracers in the boiled control extract.

The implication that much of the *myo*-inositol was metabolised to other products did not however account for the much greater efficacy of L-inositol as a quebrachitol precursor *(Table 3)*. As was always the case, the L-inositol content of the leaflets greatly exceeded the *myo*-inositol content, and calculation indicated that given complete equilibration of the tracer with the inositols already in the leaflets, the conversion of L-inositol to quebrachitol was four hundred times greater than the overall conversion of *myo*-inositol to the same product.

Incubation of ^{14}C-bornesitol with Latex in vitro

The experiments with detached leaflets indicated that there was no significant conversion of bornesitol to quebrachitol. It was however possible that the *in vivo* utilisation of bornesitol was blocked by permeability barriers hindering its transport from cell to cell. In view of the accumulation of quebrachitol in latex, and hence the likelihood that latex is a major site of quebrachitol synthesis, it seemed that effective conversion might yet be achieved by incubating bornesitol with latex *in vitro*.

In the event, 5 h incubations of ^{14}C-bornesitol (1 mg, 2.5 x 10^5 dpm) with fresh greenhouse latex yielded quebrachitol fractions containing not more than 1% of the total radioactivity: their low specific activity (<3000 dpm per milligramme quebrachitol) made it impracticable to monitor the $^{14}CO_2$ output from the quebrachitol peaks separated by GLC, but from past experience it was probable that much even of this low activity was again due to contamination with the original tracer.

DISCUSSION

In the present work with *Hevea,* GLC analysis provided estimates of the weight and ^{14}C-content of each inositol extracted from the leaflets. Assuming rapid equilibrations of radioactive tracers with the inositols already in the tissue, this allowed calculation of the rate at which each precursor was metabolised. The results indicated that the conversions of *myo*-inositol to L-inositol (300 nmoles per half-leaflet per 24 h) and of L-inositol to que-

brachitol (700 nmoles per half-leaflet per 24 h) were several hundred times faster than the reactions of the bornesitol pathway (0.3–2 nmoles per half-leaflet per 24 h). It follows that in *Hevea* leaflets, as in *Artemisia*, effectively all of the quebrachitol is formed *via* L-inositol.

An anomalous feature of the calculated data was the disparity between L-inositol formation, and the apparently faster production of quebrachitol. This discrepancy possibly implied the utilisation of existing L-inositol reserves, but could equally well arise from incomplete equilibration of the L-inositol tracer with the large internal L-inositol pool, and a consequently exaggerated estimate of quebrachitol synthesis. From a more general standpoint, however, the production of around 400 nmoles quebrachitol per half-leaflet per 24 h seemed trivial by comparison with rates in the region of 800 nmoles per millilitre per *minute* established, for example, for reactions of the glycolytic sequence in tapped latex[12]. In an attempt to put quebrachitol biosynthesis into clearer perspective, it was supposed that a typical half-leaflet weighing 1 g and containing 5 mg rubber (unpublished results) might contain 0.02 ml latex; and that this latex was entirely responsible for the synthesis of quebrachitol. This would imply the formation of about 15 nmoles quebrachitol per millilitre per *minute*.

The validity of this calculation is supported by a direct estimate of the rate of quebrachitol synthesis in trunk latex, on the basis that the quebrachitol drained from the tree by tapping is replaced as a result of the synthetic activity of the latex remaining in the drainage area. Taking as representative values a 1% weight/volume concentration of quebrachitol in S/2.d/2 latex, an average output of 35 ml latex per tapping, and a 200 ml latex content of the corresponding drainage area (determined for three typical trees under routine S/2.d/2 tapping[12]), the implied replacement of 350 mg quebrachitol per 200 ml latex per two days would require the synthesis of an average 3.1 nmoles per millilitre per *minute*. This is even lower than the value inferred for leaflet latex, and leads to the unexpected conclusion that although quebrachitol is the main water-soluble constituent of the latex, its formation possibly represents a quantitatively minor aspect of latex metabolism.

The quantitative (97%–101%) recovery of radioactivity in the inositol fractions from leaflets fed ^{14}C-L-inositol indicates that in contrast to *myo*-inositol there was no rapid metabolism of L-inositol (or quebrachitol) to products insoluble in aqueous ethanol, or to acidic or basic metabolites. This conforms to the accepted but hitherto unsubstantiated view that quebrachitol is a metabolic end-product; while the disparity between quebrachitol production and the calculated rate of rubber biosynthesis (500 nmoles isoprene unit per millilitre latex per *minute*[12]) is in any case at variance with an earlier suggestion that the similarity in taxonomic distribution might imply a direct involvement of quebrachitol in the production of polyisoprene[2].

CONCLUSION

Gas-liquid chromatographic separation together with recovery of the effluent $^{14}CO_2$ permits estimation of the content and specific activity of individual inositols in extracts from leaflets previously fed with radioactive precursors. The results show that the reaction sequence: *myo*-inositol $\dashrightarrow$ L-inositol $\dashrightarrow$ que-

brachitol takes place at rates several hundred times faster than a possible alternative pathway involving D-(–)-bornesitol, and it is concluded that in *Hevea* almost all of the quebrachitol arises by methylation of L-inositol.

The inferred rate of quebrachitol biosynthesis is very low when compared, for example, with the rate at which latex synthesises rubber. On present evidence, it appears that although quebrachitol is the predominant water-soluble component of *Hevea* latex, its formation may be a quantitatively minor feature of latex metabolism.

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