tion of many carotenoids during development of the orange color in the rind of navel oranges (*7*). The fact that lycopene accumulation was not reduced by gibberellic acid in Valencia oranges indicates that gibberellic acid does not interfere with the sequential pathway from phytoene to lycopene.

The oxy analog of CPTA caused citrus rind tissue to accumulate lycopene, but, because 29 additional analogs were not effective, a high degree of structural specificity for induction of lycopene accumulation appears to exist among the members of this class of compounds.

We also applied CPTA (5000 ppm) to fruits of apricot, prune, and several varieties of peach, to four strains of tomatoes which do not normally produce lycopene, and to roots of carrot and sweet potato. In all of the foregoing material, lycopene accumulated as a major carotenoid constituent. Similar effects were obtained when mycelia of *Blakeslea trispora* and *Phycomyces blakesleeanus* were grown in media containing 15 ppm of the compound. The quantity of lycopene that accumulated varied from a low of 10 μg per gram (dry weight) of sweet potato root to a high of 4300 μg per gram (dry weight) of *Blakeslea trispora* mycelium. The latter concentration equals or exceeds the quantities found in red varieties of tomato. Whether the difference in magnitude of response observed in treated tissues is related to different degrees of penetration or to other factors was not determined. Apple and pear fruit tissue did not accumulate lycopene in response to the compound.

We know of only one report of success with chemicals in causing an appreciable change in lycopene accumulation by plant tissues. Knypl (*8*) reported that detached pumpkin cotyledons accumulate lycopene when treated with (2-chloroethyl)trimethylammonium chloride (Cycocel). We have not seen similar activity when Cycocel was applied to citrus fruits. Since Cycocel has been widely investigated and since lycopene accumulation has not been reported for other plant tissues, it appears that CPTA, and possibly its oxy analog, are active on a much broader spectrum of plants, including at least two fungi.

The fact that a wide array of carotenogenic tissues accumulate lycopene when treated with CPTA should provide an opportunity for examination of carotenoid biosynthetic pathways and gene control mechanisms. Also, it is possible that other isoprenoid pathways are influenced by the compound.

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Trisporic Acid Synthesis in Blakeslea trispora

Abstract. *Cultures of the (−) strain of* Blakeslea trispora *synthesize trisporic acid C, a sex hormone, from precursors made by cultures of the (+) strain. The precursors are nonacidic compounds isolated from the culture medium of the (+) strain by chloroform extraction. Actidione does not inhibit trisporic acid synthesis from isolated precursors, suggesting that the enzymes involved in the synthesis in cultures of the (−) strain are constitutive.*

Trisporic acids, oxidized unsaturated derivatives of 1,1,3-trimethyl-2-(3-methyloctyl)cyclohexane (*1*), stimulate carotenogenesis in *Blakeslea trispora* (*2–5*) and the formation of zygophores (sex cells) in *Mucor mucedo* (*5–8*). Trisporic acids have been isolated from combined cultures of (+) and (−) strains of *B. trispora* (*1–7*) and *M. mucedo* (*8*) but not from individual (+) and (−) mating-types of these species. Sutter and Rafelson (*3*) reported that synthesis of trisporic acids in combined cultures of (+) and (−) strains of *B. trispora* was proportional to the amount of inoculum of the (+) strain used with excess inoculum of the (−) strain and was largely independent of the amount of inoculum of the (−) strain used with excess inoculum of the (+) strain. They suggested that cultures of the (+) strain made trisporic acids when stimulated by cultures of the (−) strain. Also van den Ende (*5*) observed that trisporic acids were synthesized when *Zygorhynchus moelleri* (a homothallic species) was incubated with cultures of the (+) strain of *B. trispora* but not with those of the (−) strain. I report, however, that cultures of the (−) strain of *B. trispora* synthesized trisporic acid C from precursors made by cultures of the (+) strain.

Cultures (3) were grown in a medium (PGT) composed of 1.25 percent potato extract, 2 percent glucose, and 0.0002 percent thiamine-hydrochloride in 500-ml flasks capped with cotton plugs (*9*) by incubation in darkness at 28°C on a 5-cm stroke New Brunswick gyrotory shaker at 175 rev/min. Trisporic acids were isolated from acidified, mixed culture media of (+) and (−) strains by chloroform extraction (*1*). The chloroform fraction was extracted with 4 percent sodium bicarbonate, and the bicarbonate or acid fraction (after acidification) was extracted again with chloroform. The chloroform was evaporated to dryness, and the residue (trisporic acids) was dissolved in ethanol. Trisporic acid B (maximum absorbance at 321 nm in ethanol) and trisporic acid C (maximum absorbance at 325 nm in ethanol) were separated by thin-layer chromatography on silica-gel plates (Eastman No. 6061) with a mixture of chloroform and acetic acid (*5*). All operations were performed under red fluorescent light (*10*).

The culture medium of the (+) strain stimulated the culture of (−) strain to synthesize trisporic acid C (Table 1). A 5-day old culture medium of the (+) strain, sterilized by filtration, was incubated for 12 hours with and without a 36-hour-old culture of the (−) strain. After incubation, the two media exhibited similar absorbances at 325 nm. However, when acid fractions of the media were isolated and examined, a significant difference in absorbance at 325 nm was observed; the

acid fraction from the culture medium of the (+) strain incubated with a culture of the (−) strain exhibited a higher absorbance at 325 nm. The reverse experiment [culture medium of the (−) strain incubated with and without a culture of the (+) strain] was performed at the same time, and the absorbance at 325 nm of the isolated acid fractions from both media were similar. To determine whether the increase in the absorbance at 325 nm indicated the presence of trisporic acids, the acid fractions from all four samples were filtered through Whatman No. 1 filter paper and concentrated 15-fold by bubbling with nitrogen; portions (30 μl) were subjected to chromatography and compared to authentic trisporic acids. Only the acid fraction from the culture medium of the (+) strain which had been incubated with a culture of the (−) strain exhibited a spot detectable under ultraviolet light with an R_F identical to that of trisporic acid C. The ultraviolet absorbance properties of a duplicate spot not exposed to ultraviolet light were identical to those of trisporic acid C, suggesting that the culture medium of the (+) strain contains a component or components which stimulate cultures of the (−) strain to produce trisporic acid C. Upon chromatography, all four samples exhibited spots detectable under ultraviolet light with an R_F identical to that of trisporic acid B. Spectroscopic examination of duplicate spots not exposed to ultraviolet light, however, revealed compounds with maximums at 285 and 240 nm. A shoulder at 321 nm was observed in the sample from culture medium of the (+) strain incubated with a culture of the (−) strain, suggesting that trisporic acid B was also present.

When cultures of the (−) strain were incubated in culture medium of the (+) strain, trisporic acid was detected in the acid fraction (Table 1 and Fig. 1A) and concomitant changes were observed in the neutral fraction; the absorbance at 285 nm decreased and that at 234 and 320 nm increased (Fig. 1B). Examination by thin-layer chromatography on silica gel of the neutral fraction from the culture medium of the (+) strain revealed that at least nine components were present. Examination of the neutral fraction from culture medium of the (+) strain incubated with cultures of the (−) strain revealed that the amount of the three components (R_F: 0.33, 0.49, and 0.79) with similar absorbance spectra [maximum absorbance at 296 (shoulder),

Table 1. Twelve-hour incubation of culture media with and without opposite mating-type culture. The filter-sterilized culture medium (125 ml) was from unmated cultures grown for 5 days in 200 ml of PGT. The unmated cultures were grown for 36 hours in 100 ml of PGT. After incubation, portions of the culture medium were diluted 20-fold with 0.1M tris(hydroxymethyl)aminomethane buffer (pH 7.5) for measurements; each acid fraction was dissolved in 5 ml of ethanol and portions were diluted 20-fold for measurements. Trisporic acid C was identified by thin-layer chromatography.

Filter-sterilized culture medium	Unmated cultures added	Absorbance at 325 nm		Trisporic acid C formation
		Culture medium	Acid fraction	
+ strain	None	0.28	0.06	No
+ strain	− strain	0.30	0.29	Yes
− strain	None	0.27	0.04	No
− strain	+ strain	0.27	0.06	No

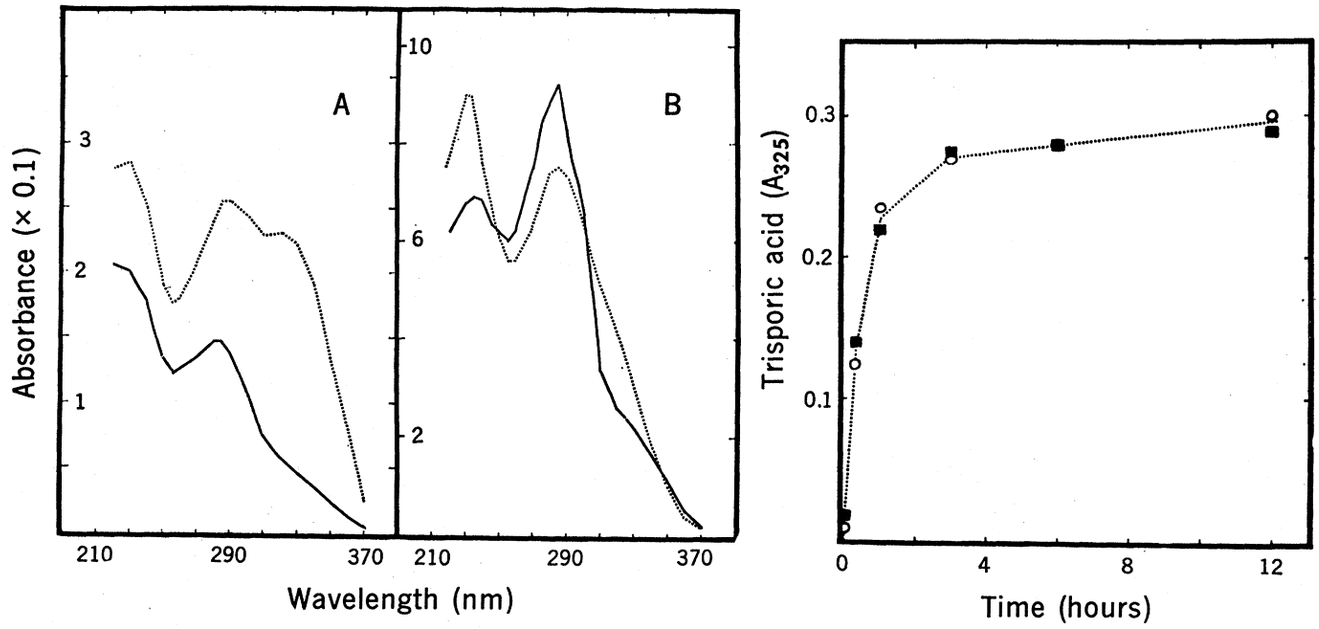

Fig. 1 (left). Ultraviolet absorbance spectra of (A) acid and (B) neutral fractions of culture media of the (+) strain incubated 12 hours with (dotted curves) and without (solid curves) cultures of the (−) strain. The conditions were as described in Table 1 except that the flasks contained 200 ml of culture medium of the (+) strain; the acid and neutral (leftovers after acids removed) fractions were dissolved in 5 ml of ethanol, and portions were diluted 35-fold for measurements. Fig. 2 (right). Kinetics of trisporic acid synthesis by cultures of the (−) strain in the presence (closed squares) and absence (open circles) of actidione. Flasks containing 36-hour-old cultures of the (−) strain in 100 ml of PGT medium received 0.75 ml of actidione (5.88 mg) or saline and 0.3 ml of neutral fraction with an absorbance at 285 nm of 505 from cultures of the (+) strain. Flasks were incubated; at indicated times acid fractions (in 5 ml of ethanol) were isolated from culture medium; portions were diluted tenfold for measurements. The difference in the absorbance at 325 nm of experimental cultures and control cultures (without actidione or neutral fraction) was used as a measure of trisporic acid formation.

285, and 275 (shoulder) nm in ethanol] diminished, suggesting that they may be precursors of trisporic acids. In addition, a new component with an R_F of 0.66 appeared in the neutral fraction with a principal maximum at 234 nm and a second maximum at about 295 nm. Separate experiments revealed that the isolated acid fraction from the culture medium of the (+) strain did not stimulate the formation of trisporic acid by cultures of the (−) strain, whereas the isolated neutral fraction was over 90 percent as effective as the total fraction.

To determine whether the neutral fraction from culture media of the (+) strain contained precursors or an inducer, or both, cultures of the (−) strain with neutral fraction from cultures of the (+) strain added were incubated with and without actidione (58 μg/ml). Trisporic acids were formed equally well in the presence and absence of actidione (Fig. 2). Control experiments in which cultures of the (−) strain were incubated with 2.5 μc of [^{14}C]leucine for 1 hour with and without actidione revealed that incorporation of leucine was inhibited over 97 percent by actidione. A similar experiment with actidione and [^{14}C]leucine has been reported (*4*). These observations suggest that (i) the culture medium of the (+) strain contains a precursor, or precursors, which cultures of the (−) strain convert to trisporic acid; and (ii) the enzymes necessary for formation of trisporic acid in cultures of the (−) strain are constitutive.

Because cultures of the (−) strain of *B. trispora* synthesize trisporic acid C from precursors made by cultures of the (+) strain, the observation (*3*) that trisporic acid synthesis was proportional to the amount of inoculum from the (+) strain used in combined cultures of the (+) and (−) strains must now be interpreted that precursor formation by cultures of the (+) strain is the rate-limiting step in trisporic acid synthesis. In a similar fashion, van den Ende's (*5*) observation that cultures of the (+) strain but not of the (−) strain of *B. trispora* produce trisporic acids when incubated with *Z. moelleri* can be interpreted that *Z. moelleri*, while being able to convert precursor to trisporic acids, is either unable to make precursor or (and more likely) synthesizes such small quantities of precursor that trisporic acid synthesis was not detected under the conditions employed. Consistent with these views is the observation (*6*) that cultures of the (−) strain of *B. trispora* but not those of the (+) strain synthesized trisporic acids or trisporone, or both, when incubated in filter-sterilized culture medium from combined cultures of (+) and (−) strains of *B. trispora.*

Dutch investigators (*5, 6*) have reported that a single trisporic acid, isolated from combined cultures of (+) and (−) strains of *B. trispora,* induced the formation of zygophores in cultures of both the (+) and (−) strains of *M. mucedo,* implying that a single mating-type produces a hormone that induces the formation of zygophores for both mating-types. In contrast, German investigators (*7, 11*) postulated that (i) separate sex hormones induce zygophore formation in cultures of (+) and (−) strains of *M. mucedo*; (ii) cultures of the (+) strain produce the hormone which induces the formation of zygophores in cultures of the (−) strain; and (iii) cultures of the (−) strain produce the hormone which induces the formation of zygophores in cultures of the (+) strain. It should be possible to determine which of the above two hypotheses is correct—that is, determine whether trisporic acids made by a single mating-type induce zygophores in both mating-types or in only the opposite mating-type—by utilizing the extract of trisporic acids isolated from cultures of the (−) strain.

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Bulk Isolation in Nonaqueous Media of Nuclei from Lyophilized Cells

Abstract. *Intact lyophilized nuclei are obtainable from a variety of tissues, either in situ or in culture, by freezing at −156°C, drying at −25°C, and mechanical disassociation in glycerol at 2°C. Centrifugal separation of nuclei is accomplished in an 85 : 15 by volume mixture of glycerol and 3-chloro-1,2 propanediol at 2°C. The method gives homogeneous nuclear preparations in high yield with preservation of labile and water-soluble constituents.*

Cell fractionation for nuclear isolation is most conveniently processed in aqueous media (*1*). Conventional homogenization of isolated tissue in aqueous media, however, introduces unavoidable analytical artifacts rendering assays for many important biological materials invalid. Catabolic events associated with anoxia, extraction of water-soluble nuclear constituents, and cross-contamination between nuclei and cytoplasm compromise aqueous nuclear preparative methods (*2*). Despite these deficiencies, nuclear isolation in water remains a standard procedure, since alternative nonaqueous preparative methods have been cumbersome and destructive of both nuclear morphology and enzymatic activity (*3*). Microdissection of nuclei from individual lyophilized cells circumvents these difficulties, but is tedious, applicable only to large nuclei, and necessarily of low yield. This report describes a bulk method, with preliminary steps based on the system of Lowry (*4*) for lyophilization of tissue sections, but extended and adapted to permit efficient isolation of remarkably clean, lyophilized nuclei in high yield.

Though technical maneuvers involved in nonaqueous nuclear isolation methods are similar, certain modifications are critical for reproducible results. Tissue or cells must be frozen rapidly, not only to minimize ice artifacts, but to limit redistribution of diffusible intracellular components and degradation of