thicknesses of volcanic deposits on Mars increase in younger eruptions. This trend could reflect changes in styles of eruption, changes in magma composition or viscosity, or other effects. In general, higher rates of eruption and magmas that are more mafic in composition produce longer, thinner flows. Thus, the relatively thin volcanic deposits in the Noachian and Early Hesperian epochs could reflect highly fluid lava flows that spread over large areas and initially ponded in low-lying regions within the heavily cratered terrain. From geochemical considerations, Burns and Fisher (*19*) suggested that ultramafic lava flows such as komatiites may be present on Mars. On Earth, komatiites are considered to have been extremely fluid and erupted rapidly at high temperatures, characteristics that are consistent with the thin lava flows suggested in early Mars' history.

Comparison of magma production on Mars with Earth and Venus (Table 2) shows that extrusive (volcanic) production rates appear to be a function of planetary mass; Mars has the lowest rate (0.018 km^3/yr). Differences in planetary interiors and styles of tectonism make selection of intrusive-to-extrusive ratios difficult. For simplicity of comparisons, the same 8.5:1 ratio derived from Earth and used for Mars was also applied to Venus.

Results for total magma production rates following accretion and formation of a stable crust (Table 2) also scale with planetary mass. However, even when scaled to Earth's mass and production rate, the magma production on Mars is significantly lower than for the other terrestrial planets. Compared to the Moon (normalized to Earth), lunar magma production appears anomously high, and rates for both extrusive and total magma production are greater than values for Mars, despite the much smaller size of the Moon. This comparison suggests that magma generation on the Moon may have been affected by processes or factors such as tidal stresses by Earth, similar to models applied to outer planet satellites that experience volcanism (*23*).

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24. This study was supported by the Office of Planetary Geology, National Aeronautics and Space Administration. We thank E. Lo, L. Bolef, and the late T. Townsend for computer programming. J. McHone, J. Crisp, and an anonymous reviewer provided helpful reviews of the manuscript.

18 July 1991; accepted 23 September 1991

Indole-3-Acetic Acid Biosynthesis in the Mutant Maize *orange pericarp*, a Tryptophan Auxotroph

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The maize mutant *orange pericarp* is a tryptophan auxotroph, which results from mutation of two unlinked loci of tryptophan synthase B. This mutant was used to test the hypothesis that tryptophan is the precursor to the plant hormone indole-3-acetic acid (IAA). Total IAA in aseptically grown mutant seedlings was 50 times greater than in normal seedlings. In mutant seedlings grown on media containing stable isotope-labeled precursors, IAA was more enriched than was tryptophan. No incorporation of label into IAA from tryptophan could be detected. These results establish that IAA can be produced de novo without tryptophan as an intermediate.

THE PLANT HORMONE AUXIN, OR INDOLE-3-ACETIC ACID (IAA), has been studied for more than 100 years (*1*), yet it remains unclear how the principal endogenous auxin is synthesized. The amino acid tryptophan is considered to be a precursor to IAA in plants because of structural similarities and because it appears to be the precursor in bacteria (*2*) and in plant cells transformed by *Agrobacterium tumefaciens* (*3*). Efforts to characterize the intermediates and enzymes involved in IAA biosynthesis have established that plants are competent to synthesize IAA from tryptophan by several different pathways (*4*). Nevertheless, microbial contamination, cellular compartmentation, and possible multiple pools of precursors have combined to make the data ambiguous.

Relatively little tryptophan is converted to IAA in sterile plant material (*5*). In an auxin bioassay with *Avena* coleoptiles, there is no growth response to tryptophan under sterile conditions, although anthranilic acid is active (*6*). In *Lemna gibba*, D-tryptophan is not converted to IAA, and the rate of conversion from L-tryptophan is far lower than would be expected for a direct precursor (*7*).

We have done a biochemical analysis of mutant plants that are incapable of making tryptophan to determine whether tryptophan is a precursor to IAA. One of the problems with producing plant amino acid auxotrophs is gene redundancy. A conditional tryptophan auxotroph of *Arabidopsis thaliana*, with a mutation in the tryptophan synthase (E.C. 4.2.1.20) B subunit (*trpB*) gene, contains a second gene encoding *trpB* activity (*8*). The expression of this second, nonmutated, gene would limit the utility of this mutant for studies of auxin metabolism, because the requirements for hormone precursor are expected to be low relative to other uses for tryptophan. Maize also has two *trpB* genes (*9*). We describe here the analysis of IAA biosynthesis in a maize tryptophan auxotroph, *orange pericarp* (*orp*); this phenotype results from recessive mutations in both unlinked *trpB* loci (*10*).

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We determined the in vivo amounts and precursor labeling of both tryptophan and IAA in individual seedlings grown from embryos removed (using aseptic technique) from maize kernels 30 days after pollination (*11*). Quantities of IAA and tryptophan in mutant and normal seedlings were determined by isotope dilution analysis and quantitative mass spectrometry (*12*). If tryptophan were the precursor to IAA, IAA amounts in a tryptophan auxotroph would be reduced; however, the amount of IAA (*13*) in 10-day mutant seedlings was approximately 50-fold greater than in normal seedlings (Fig. 1A). Amounts of free tryptophan (*14*) were reduced in mutant seedlings (Fig. 1B). These results are consistent with synthesis of IAA from a heretofore uncharacterized branch point off the indole pathway (*15*), before the synthesis of tryptophan. Addition of tryptophan to the medium did not alter IAA amounts in the mutant or normal seedlings, indicating that tryptophan is not an immediate precursor to IAA (Fig. 1A).

In auxotrophs, precursors before the block in the biochemical pathway build up and can drive branch reactions. These *orp* seedlings do accumulate precursors as shown by their blue fluorescence, characteristic of anthranilate glycoside, and the *orp* color is related to excess indole (*10*).

High amounts of total IAA in mutant seedlings suggest that IAA biosynthesis diverges from the tryptophan pathway before the indole-serine condensation step catalyzed by *trpB*. Additional evidence for such a branch point was obtained with the use of isotopic labeling. These experiments were designed to overcome problems caused by compartmentation of indole metabolism primarily in the plastids (*7, 16*). D_2O, a "totally invasive label," was used to

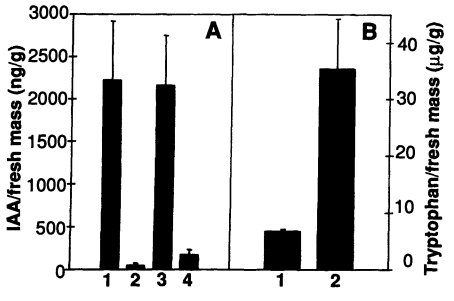

Fig. 1. Amounts of total (*13*) IAA (**A**) and free (*14*) tryptophan (**B**) in *orp* and normal 10-day maize seedlings as determined by quantitative GC-MS analysis. (A) Lane 1, *orp* seedlings; lane 2, normal seedlings; lane 3, *orp* seedlings; and lane 4, normal seedlings. Seedlings in lanes 3 and 4 were grown with exogenous tryptophan. (B) Lane 1, *orp* seedlings; and lane 2, normal seedlings. Values are the average ± SE of three (B) or four (A) determinations.

Table 1. Incorporation of deuterium into stable ring positions of tryptophan (Trp) and IAA in normal and *orp* seedlings. Embryos were grown for 6 days on water media, then 8 days on 30% D_2O. Indole formed after tryptophanase treatment of tryptophan or methylated IAA were analyzed by GC-MS. Data are from individual seedlings and the values presented are typical (*20*). Values reported are increased enrichment due to the D_2O treatment (*21*) and are expressed as percent relative to the total abundance of ions at *m/z* 117 to 123 or *m/z* 130 to 136.

Plant	Trp *m/z*(%) 118–123	IAA *m/z*(%) 131–136	$\frac{[^2H]IAA}{[^2H]Trp}$
	Normal		
1	34.8	26.7	0.77
2	21.1	10.4	0.49
	orp		
3	16.0	27.4	1.71
4	18.4	24.0	1.30

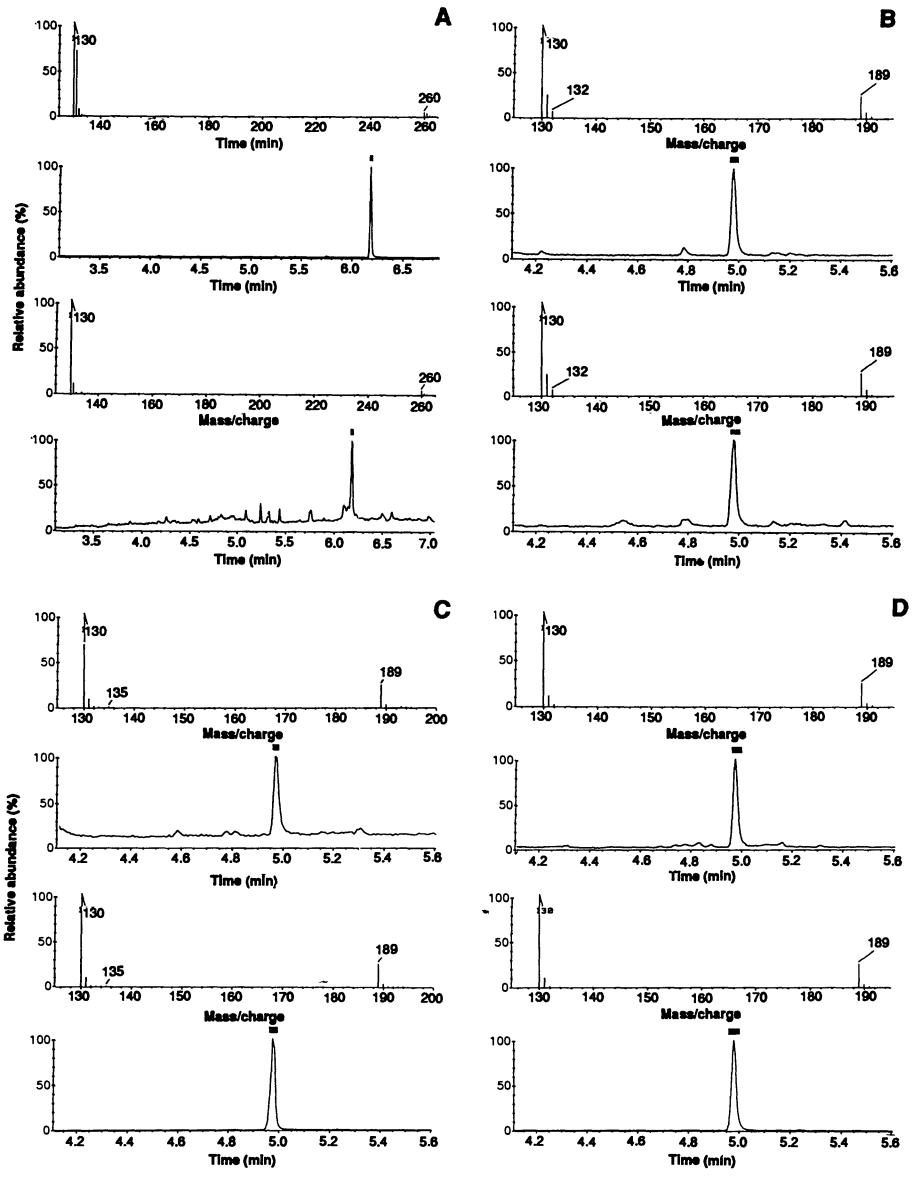

Fig. 2. Average spectra and total ion chromatograms (TIC; the sum of monitored ions) of tryptophan and IAA from 10-day-old seedlings. In each panel the top set of data are for normal seedlings and the bottom set are for the mutant seedlings. Data presented in (**A**) and (**B**) are from seedlings grown on agar containing 30 μM [^{15}N]anthranilic acid. For tryptophan analysis (A), ions at the quinolinium ion region (*m/z* 130 to 135) and at the molecular ion region for the *N*-acetyl methyl ester derivative (*m/z* 260 to 265) were monitored, and for IAA methyl ester (B) the ions at *m/z* 130 to 135 and 189 to 194 were monitored. Bar over peak on TIC indicates region of averaged spectra shown in frame above. Incorporation of ^{15}N from anthranilic acid into tryptophan results in an increase at *m/z* 131 and 261 and for IAA an increase at *m/z* 131 and 190. In (**C**) and (**D**) are spectra and TIC of methyl IAA isolated from normal and *orp* seedlings after 10 days growth on 166 μM L-[^{15}N] or L-[$^2H_5$]tryptophan. Although use of L-[$^2H_5$]tryptophan (C) gives greater sensitivity for analysis, confirmation was obtained with the use of L-[^{15}N]tryptophan (D). Each experiment was repeated four to six times and the data presented are individual GC-MS analyses of representative samples.

label early indolic precursors for which enol-diol equilibration allows exchange, that is, phosphoenol pyruvate and erythrose-4-phosphate before they enter into the shikimic acid pathway (*17*). As a general label, D_2O also has the advantage that specific knowledge of the biosynthetic pathway is not required.

Both mutant and normal seedlings incorporated deuterium into ring positions of IAA to a similar extent (Table 1). Mutant seedlings, however, incorporated much less deuterium into the indole ring of tryptophan than did the normal seedlings. In the *orp* seedlings the isotopic enrichment of the IAA pool was greater than the enrichment in the tryptophan pool. The greater enrichment of product (IAA) relative to the enrichment in the purported precursor (tryptophan) indicates that a nontryptophan pathway to IAA functions in these seedlings.

The use of ^{15}N-labeled anthranilic acid to label the indole pathway resulted in labeled tryptophan in normal seedlings, but no detectable incorporation into tryptophan in *orp* seedlings (Fig. 2, A and B). Note the high incorporation of ^{15}N into tryptophan of the normal seedlings and no significant incorporation in the *orp* seedlings (Fig. 2A; the abundance of 131 and 261 is not increased over the normal isotope abundance). Both mutant and normal seedlings incorporated similar amounts of [^{15}N]anthranilate into IAA. The major difference between normal and *orp* seedlings was that the IAA pool was more enriched than the tryptophan pool in the mutant. Feeding L-[^{15}N]tryptophan or L-[$^2H_5$]tryptophan at amounts higher than necessary to benefit the growth of *orp* seedlings (*18*) resulted in no detectable incorporation of label into IAA (Fig. 2, C and D).

The formation of IAA from tryptophan was first noted by Wildman *et al.* in 1946 (*19*); the idea that IAA is made by the sequential deamination and decarboxylation of tryptophan has had general acceptance. The use of stable isotope labeling allows more detailed quantitative evaluation of metabolic processes in plant hormone metabolism. Quantitative arguments (7) indicated that tryptophan was an unlikely precursor to IAA, and the use of the tryptophan-requiring mutant *orp* provides evidence that ideas regarding IAA biosynthesis from tryptophan do not fully explain the biosynthesis of IAA.

Although studies with mutants have shown that it is possible for IAA biosynthesis to occur without tryptophan as a metabolic intermediate, they do not exclude a tryptophan pathway to IAA in plants. In *Lemna*, tryptophan is, at best, only a minor contributor to IAA biosynthesis (7). Failure of either L-[$^2H_5$] or L-[^{15}N]tryptophan to significantly label IAA in normal or *orp* maize suggests a similar situation exists in maize; therefore, the nontryptophan pathway is the primary route of IAA biosynthesis.

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11. The embryos used in the labeling studies were obtained from self-pollinated plants derived from a cross containing Black Mexican Sweet as the female parent. The male parent was derived from three generations of selection of superior heterozygotes from a combination of elite germplasm (Mo20Y, W23, A632, and Alexander High Oil) containing both mutant alleles. Embryos were germinated on 3 ml of MS medium [T. Murashige and F. Skoog, *Physiol. Plant.* **15**, 473 (1962)] solidified with agar. Nonsterile embryos were discarded. Two basic protocols were used. First, embryos were germinated directly on MS supplemented with 30 μM labeled tryptophan or anthranilate, or on media made from 30% D_2O and grown for 10 days under low light. In some experiments, embryos were grown under low light for 6 days, after which 0.5 ml of either D_2O or a solution containing a labeled compound was applied to the top of the agar medium. Eight days later the plants were harvested. All seedlings were weighed and frozen in liquid N_2 after harvest. Plants were stored at −80°C and shipped on dry ice.
12. K.-H. Chen, A. N. Miller, G. W. Patterson, J. D. Cohen, *Plant Physiol.* **86**, 822 (1988); high-performance liquid chromatography (HPLC) purification was done on a Nova-Pak 4-μm C_{18} RCM cartridge (Waters Chromatography Division; 5 mm by 100 mm). Gas chromatography–mass spectrometry (GC-MS) analysis was carried out on a 5890 GC connected to a 5971A mass-selective detector (Hewlett-Packard). For studies of deuterium incorporation, 14 ions were monitored at mass per unit charge (*m/z*) 130 to 136 and *m/z* 189 to 195.
13. Free, esterified, and amide IAA were determined as described [J. D. Cohen, B. G. Baldi, J. P. Slovin, *Plant Physiol.* **80**, 14 (1986); K. Bialek and J. D. Cohen, *ibid.* **90**, 398 (1989)]. Conjugated IAA accounted for nearly 99% of the total IAA in mutant seedlings, and most was in the form of amide conjugates.
14. Tryptophan was determined without hydrolysis of protein-bound tryptophan, and data reflect the free amino acid. In a young seedling, it would be expected that some free tryptophan would result from the turnover of storage proteins.
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18. The labeled compounds were supplied at a concentration of 30 μM. A beneficial effect of tryptophan could be seen at concentrations as low as 15 μM (*10*). It has not been possible to completely restore normal phenotype and rescue the mutants to reproductive maturity by supplying tryptophan.
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20. These studies were done on individual seedlings rather than pooled samples because about 1 in 30 individuals showed high incorporation values which distorted pooled sample interpretations. A small number of seedlings showed bacterial growth after freeze-killing, and thus it is possible that the higher incorporation was the result of occasional bacterial contamination.
21. Tryptophan was quantified with the use of L-[2,4,5,6,7-$^2H_5$]tryptophan as an internal standard. Tryptophan was extracted as for IAA (*12*), then purified on a 4-ml column of Dowex 50X2-400 (Sigma) and derivatized with a mixture of 2 ml of anhydrous methanol and 0.5 ml of acetic anhydride at 65°C for 1 hour to form the *N*-acetyl methyl ester. The derivatized tryptophan was purified by HPLC on a Waters 4-μm C_{18} Nova-Pak column (3.9 by 150 mm) by use of a 30% methanol to water mixture as the mobile phase. GC-MS analysis was essentially as described for IAA except that the rate of temperature increase was 30°C/min after a 1-min hold following injection at 140°C. Ions monitored were *m/z* 130, 135, 260, and 265. For analysis of deuterium incorporation, tryptophan was treated with tryptophanase (E.C. 4.1.99.1, from *Escherichia coli*) following the Dowex step and the resulting indole partitioned into pentane for analysis by GC-selected ion monitoring MS (ions at *m/z* 117 to 123). Conditions for enzyme treatment were essentially as stated by the supplier (Sigma). Data shown in Table 1 were corrected for the natural abundance of the heavy molecular species [R. M. Silverstein, G. C. Bassler, T. C. Morrill, *Spectrometric Identification of Organic Compounds* (Wiley, New York, ed. 3, 1974)].
22. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the Department of Agriculture and does not imply approval to the exclusion of other products or vendors that may be suitable.

23. Supported in part by NSF grants DMB 8811027 to M.G.N., DCB 8917378 to J.P.S. and J.D.C., and U.S.–Israel Binational Agricultural Research and Development Fund grant U.S.-1362-87 to J.D.C. This is contribution number 11,452 from the Missouri Agricultural Experiment Station journal series.

28 June 1991; accepted 30 September 1991