

the new bonanza, and in April, 1928, two months after the original strike, the town had a population of 1500, the Gilbert brothers had installed an electric light plant and numerous stores and cabins had been built. Almost before it was possible to say 'Wahmonie, Nevada,' the boom collapsed." The townsite is located at an elevation of 4300 feet on a gentle south-facing slope, on unsorted alluvium typical of fans in this area, but free of major drainageways. The soil has a gravelly, granular, sandy-loam texture with lag gravel on the surface, the whole derived from the lavas and tuffs of the adjacent mountains. The platting of the townsite apparently amounted to little more than clearing of the shrubs from the street system, but the "streets" are still conspicuous because of the peculiarity of the vegetation which has become established on them in the course of 33 years. The perennial vegetation was sampled on the streets and on the less disturbed portion of the bajada immediately adjacent, which was taken as the best available "control." A distance method similar to the quarter method of Curtis and Cottam (4) was used, but ten individuals were recorded per point. Fifty points were taken, giving a total sample of 500 individuals for each site. The results are presented in Table 1.

Clearly, there have been pronounced changes in the physiognomy and floristic composition of the vegetation in Wahmonie. In fact, an open stand of bunch-grass has partially replaced the desert shrub community, because of the large increase of *Stipa*. On the whole, invasion by shrubs has been slow, and the species composition has changed both quantitatively and qualitatively. Of the dominant shrubs on this portion of the bajada, only *Ephedra* and *Lycium* are present on the townsite in large numbers, and it happens that both of these are species which sprout vigorously from underground parts when the tops are destroyed. It is significant that *Thamnosma*, *Hymenoclea*, and *Sala-zaria*, which are characteristic shrubs of naturally disturbed desert habitats, such as drainageways and actively eroded bedrock areas, should appear in large numbers on a man-disturbed site. This establishes a pioneer or weedy character for certain plants of dry washes, which have been regarded as an *ad hoc* climax. Shreve (2) concluded: "Each habitat in each subdivision of a desert area has its own climax, which must not be given an elastic definition and must be inter-

preted as having a genetic relation to any other climax." Since the word climax, as applied to vegetation, conveys an idea of relative stability, it seems scarcely applicable in the case of desert washes, which are periodically scoured by floods carrying great quantities of coarse detritus. The average wash presents an extensive open surface favorable for invasion by pioneer plants possessing the requisite adaptations for efficient seed dispersal, celerity of growth, and early maturation. One might suppose that all plants of this habitat have a greater water requirement than plants of upland sites and for this reason are restricted to naturally irrigated areas of the desert. However, the present study shows that several shrubs of dry washes can become established in abundance on the more xeric upland portions of an alluvial fan when the competition of the dominant upland shrubs is largely removed. Obviously, these pioneer plants of the desert play a role similar to that of successional plants of more humid regions (5).

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References and Notes

1. C. H. Muller, *Ecology* 21; 206 (1940).
2. F. Shreve, *Carnegie Inst. Wash. Publ. No. 591* (1951).
3. N. Murbarger, *Ghosts of the Glory Trail* (Desert Magazine Press, Palm Desert, Calif., 1956).
4. G. Cottam and J. T. Curtis, *Ecology* 37; 451 (1956).
5. This report is based upon investigations at the Nevada Test Site performed under contract No. AT(29-2)517 between the U.S. Atomic Energy Commission and New Mexico Highlands University.

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Biosynthesis of Stigmasterol in Tomato Fruits

Abstract. The presence of stigmasterol in tomato fruits was demonstrated. Labeled mevalonic acid was incorporated into this sterol, while sodium acetate was not. The identity of the isolated product was established by rigorous purification to constant specific activity, melting point determinations, and infrared spectrum.

The biosynthesis of steroids in plants has only recently begun to receive attention, in contrast to the large volume of work on the biosynthesis of animal steroids (1). In 1958 Sander and Grisebach (2) reported the incorporation of labeled acetate into tomatin in tomato seedlings. Guseva *et al.* (3) have studied the biosynthesis of the stero-

Table 1. Purification and radioactivity of stigmasterol. Melting points were taken on a Kofler block and are corrected. Radioactivity was determined to ± 0.9 error.

Treatment	mp ($^{\circ}$ C)	Count/min mmole
<i>Stigmasterol acetate</i>		
A	142-144	3200
B	142-145	1870 \pm 90
C	143-145	1280 \pm 70
D	143-145	913 \pm 68
E	143-145	840 \pm 68
<i>Stigmasterol</i>		
F	168-170	733 \pm 49
G	168-170	766 \pm 58
H	169-171	770 \pm 54

idal alkaloids chaconine and solanine in potato sprouts, finding that mevalonic acid is no more effective as a precursor than acetate. Ramstad and Beal (4) injected labeled mevalonic acid into *Digitalis lanata* plants, from which they then extracted radioactive digitoxigenin. On feeding radioactive mevalonic acid or sodium acetate to *D. purpurea* plants, Gregory and Leete (5) found that, while both were incorporated into digitoxigenin, only the acetate was incorporated into the side chain. Recently Nicholas (6) showed that β -sitosterol is synthesized from labeled mevalonic acid by *Salvia sclarea*; the sterol from the flowers had higher radioactivity than that from other parts of the plant.

In our first work on the biosynthesis of plant steroids (7), we incubated *Dioscorea floribunda* tuber homogenates with labeled acetate and mevalonate; acetate was incorporated into diosgenin, but mevalonate was not. While it is probable that the steroid nucleus is formed in the same manner in plants and animals, more information is needed concerning the origin of the side chain in typical plant steroids. The present study was initiated by a separate investigation of the biosynthesis of carotenoids in *Lycopersicon esculentum* fruits from mevalonic acid (8), in which a highly radioactive sterol fraction was discovered.

Preliminary experiments were carried out on "Snowball" tomatoes (9). By injection of radioactive mevalonic acid by the technique described by Purcell *et al.* (8) and use of the separation procedure described by Heftmann *et al.* (10), we isolated pure stigmasterol, which had an infrared spectrum identical with that of an authentic sample. The yield of the sterol fraction was low, however, and the radioactivity was not high enough to allow dilution with carrier material. Accord-

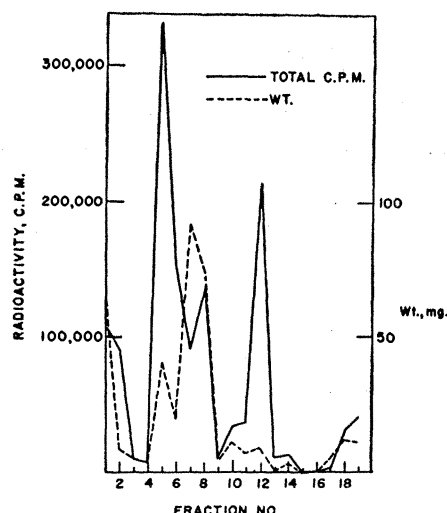


Fig. 1. Distribution of radioactivity in chromatographic fractions.

ingly, in all further work red tomatoes were used.

Thirty-one red tomatoes, weighing 4714 g, were injected with 2 μ C each of mevalonic acid-2- C^{14} . After 24 hours, they were homogenized in a Waring blender with an equal weight of water, hydrolyzed by refluxing with concentrated hydrochloric acid (150 ml/lit.), and extracted with dichloromethane. The extracts were washed with 2N sodium hydroxide and evaporated. The residue was refluxed with phthalic anhydride in pyridine and the hemipthalates of the alcoholic material were isolated by extraction with base. Hydrolysis of the hemipthalates by refluxing with sodium methoxide solution gave 865 mg of a crude alcoholic fraction. This material was chromatographed on 30 g of alumina (11), grade III, taking the following 100-ml fractions: 1 to 2, 10-percent, 3 to 4, 25-percent, and 5 to 6, 50-percent benzene in petroleum ether; 7 to 9, benzene; 10 to 11, 10-percent, 12 to 13, 25-percent, and 14 to 15, 50-percent ether in benzene; 16, ether; 17, 1-percent, 18, 5-percent, and 19, 20-percent methanol in ether. A 0.1-mg aliquot of each fraction was plated on a copper planchet and the radioactivity was determined under a micromil window tube in an atmosphere of Q gas. The distribution of radioactivity in these fractions is shown in Fig. 1. Fractions 7 and 8 contained the sterol mixture, which proved much more difficult to purify than in the case of the white tomatoes. After preliminary chromatographing and crystallization, the material that melted above 140°C was combined and acetylated, and the acetates (42 mg) were diluted with 42 mg

of pure stigmasterol acetate. This material was purified by chromatography, crystallization from acetic acid, two more chromatographic separations, and recrystallization from methanol. At this point it weighed 8.2 mg, melted at 139° to 141°C, and had a specific activity of 17.8 count/min per milligram. After dilution with 9.6 mg of pure stigmasterol acetate, it was subjected to the following operations, as illustrated in Table 1. After each treatment, 2-mg aliquots were counted in duplicate to a 0.9-level of confidence. Treatment A was recrystallization from acetic acid. Treatments B, C, D, and E consisted of successive recrystallizations from methanol. The material from E was hydrolyzed with sodium methoxide in methanol, and the product (F) was recrystallized from methanol (G), and again from ethanol (H). These treatments gave stigmasterol having a constant specific activity of 770 count/min per millimole.

In another experiment, tomato fruits were injected with sodium acetate-2- C^{14} and worked up as before. In this case the sterol fraction was much less radioactive, and the pure stigmasterol finally obtained showed no significant radioactivity.

The presence of sterols in tomato fruits has not been reported heretofore and much remains to be learned about the biosynthesis of sterols in fruits. Unfortunately, the level of radioactivity of the stigmasterol obtained in these experiments was too low to permit degradation for establishing the pattern of labeling. In contrast to our previous findings on the biosynthesis of diosgenin in a tuber (7), it is now evident that mevalonic acid is a precursor of stigmasterol in tomato fruit. Acetate is apparently utilized so extensively for other biochemical reactions that only insignificant quantities are incorporated into stigmasterol under the conditions of our experiment.

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References and Notes

1. E. Heftmann and E. Mosettig, *Biochemistry of Steroids* (Reinhold, New York, 1960).
2. H. Sander and H. Grisebach, *Z. Naturforsch.* 13b, 755 (1958).
3. A. R. Guseva, M. G. Borikhina, V. A.

Paseschnichenko, *Biokhimiya* 25, 282 (1960); *Doklady Akad. Nauk S.S.S.R.* 133, 228 (1960).

4. E. Ramstad and J. L. Beal, *J. Pharm. and Pharmacol.* 12, 552 (1960).

5. H. Gregory and E. Leete, *Chem. & Ind. (London)* 1960, 1242 (1960).

→ H. J. Nicholas, *Nature* 189, 143 (1961).

→ E. Heftmann, R. D. Bennett, J. Bonner, *Arch. Biochem. Biophys.* 92, 13 (1961).

8. A. E. Purcell, G. A. Thompson, Jr., J. Bonner, *J. Biol. Chem.* 234, 1081 (1959).

9. Grown from seeds generously supplied by A. E. Thompson, Department of Horticulture, University of Illinois, Urbana, Illinois, at the Plant Industry Station of the U.S. Department of Agriculture, Beltsville, Maryland, through the courtesy of Joseph R. Haun, New Crops Research Branch.

→ E. Heftmann, B. E. Wright, G. U. Liddel, *Arch. Biochem. Biophys.* 91, 266 (1960).

11. Nonalkaline, from M. Woelm, Eschwege, Germany.

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Chemical Analysis of Surfaces by Use of Large-Angle Scattering of Heavy Charged Particles

Abstract. The Rutherford scattering of charged particles from the heavier elements and nuclear scattering and (α, p) reactions from the light elements result in energy spectra that are characteristic of the nucleus being bombarded. A simple apparatus for analyzing surfaces based on these ideas can be made by using an alpha source such as Cm^{244} , a solid state detector, and an electronic pulse height analyzer.

New methods of chemical analysis are always of interest, especially if they can be made more automatic than conventional techniques. This report calls attention to a nondestructive method, best used in vacuum, that is particularly applicable to the study of surfaces (the top 1 to 100 μ). The apparatus is very simple and the information is obtained in electronic form, which recommends the method especially for application at distances where the transmission of data is a problem.

It is proposed that scattering of a monochromatic, collimated beam of charged particles (for example, alpha particles from a thin radioactive source) be utilized for analysis of a solid. This method of analysis has been mentioned before (1), but the application of the newly developed solid state detectors makes it particularly attractive to both solid and particulate analysis.

For relatively low energy particles, such as alpha particles from the usual radioactive sources, and for those elements that are heavier than aluminum, the large-angle scattering of heavy charged particles is primarily Rutherford scattering. The energy of such scattered particles from a target thin enough so that the energy of the par-