the powdered sample in an Erlenmeyer flask. The flask was shaken well and allowed to stand for ten minutes, after which the solution was immediately filtered clear into a 100-ml. volumetric flask. Five milliliters of ferric nitrate reagent was added and distilled water was added to volume. The absorbance of the solution was then read at 525 mμ when blanked against 20% ethyl alcohol. The amount of free salicylic acid present was read from the standard curve and the per cent labeled amount recorded.

In the case of formulation A the solution became turbid on making it up to volume with distilled water. The turbidity was probably due to the dissolved magnesium stearate being thrown out of solution by water. The solution was filtered before reading the absorbance.

The hardness of the tablets as determined by the Strong-Cobb tablet hardness tester was found to be A, 7 Kg.; B, 10 Kg.; C, 9 Kg.; and D, 10 Kg. The disintegration time of the freshly prepared tablets was found to be A, fifteen seconds; B, twenty seconds; C, ten minutes; and D, twelve minutes.

The per cent labeled amount of A, B, C, and D at the three temperature levels was determined weekly throughout the period of study and these results are recorded in Tables II and III.

DISCUSSION

Although it is stated that aspirin forms a pasty mass when in contact with acetophenetidin (1), the diluents in formula B are obviously sufficient to hinder this reaction. It has also been reported that the deterioration of aspirin (1) is hindered by sugar and glycerol, and this may help to explain the stability of this formulation.

Formulation A had a lower initial value than formulations B, C, and D. The lower value is probably due to the presence of magnesium stearate in the formulation. This is in agreement with the report of Ribeiro, *et al.* (2). Magnesium stearate was used as a lubricant because talc, liquid paraffin, or a combination of the two did not slug well.

Formulation A did not deteriorate when kept under refrigeration. At room temperature it deteriorated slowly, and the final value after a five week aging period was 98.8% of the labeled amount. At 45° decomposition of aspirin was more rapid. There was a distinct odor of acetic acid from the second week onward, and the final value was 84.0% of the labeled strength.

Formulation B showed no deterioration under refrigeration and room temperature. At 45° there was slight decomposition in that the final value was 99.0% of the labeled amount.

Formulation C also showed no apparent deterioration under refrigeration and room temperature. At 45° there was a very slight deterioration and the tablets took on a faint buff color.

Formulation D showed hardly any deterioration under refrigeration and room temperature. At 45° it also decomposed very slowly to a final value of 98.7% of the labeled amount and the tablets showed a slight discoloration.

Taking appearance, disintegration time, and stability of aspirin into consideration, formula B emerges as the best of the four formulations studied and can easily be used in the preparation of tablets of aspirin, phenacetin, and caffeine.

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Incorporation of 2-C^{14}-Sodium Acetate into Sterol and Pentacyclic Triterpenes of *Salvia officinalis*

By HAROLD J. NICHOLAS

C^{14} from 2-C^{14}-sodium acetate presented to *S. officinalis* L. by absorption into isolated leaves and into flowering stems devoid of leaves was incorporated into both sterol and acidic triterpenes of each plant section.

IN STUDYING the incorporation of precursors into sterols and triterpenes of higher plants it would be of interest to know whether all organs of the plant possess this synthetic capacity. At our present state of knowledge such information is not available in the literature, especially in regard to biosynthesis of the pentacyclic triterpenes (1). The present study was conducted primarily to ascertain if isolated organs of *S. officinalis* L. would incorporate 2-C^{14}-sodium acetate into sterol (β-sitosterol) and pentacyclic triterpenes (oleanolic acid and ursolic acid I). These substances have previously been reported present in *S. officinalis* L. (2, 3). Presenting labeled precursors to higher plants quantitatively frequently poses a problem. For our preliminary work we

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TABLE I.—INCORPORATION OF 2-C^{14}-SODIUM ACETATE INTO CRUDE STEROL AND TRITERPENE FRACTIONS OF *S. officinalis* ORGANS

Plant Organ	Crude Sterol Fraction		Crude Triterpene Fraction			
	C^{14} Content		Ursolic Acid I		Oleanolic Acid	
	Wt., mg.	c. p. m.	Wt., mg.	C^{14}, c. p. m.	Wt. mg.	C^{14}, c. p. m.
Flowers and stem	2.2	26,950	2.5	953,600	2.5	99,200
Leaves only	2.0	13,750	0.8	965,800	2.2	203,960

have chosen the method of Krotkov and Barker (4), who studied the distribution of radioactivity into various fractions following the absorption of solutions of C^{14}-labeled acetate into cut sections of tobacco leaves while stimulated by light. This method has the advantage of permitting quantitative absorption of the solutions to be absorbed, and in a relatively short period of time.

EXPERIMENTAL

Materials and Methods.—All solvents were A. R. grade or equivalent. Sterol and triterpene samples for dilution were from sources previously described (5). The 2-C^{14}-sodium acetate was purchased from Isotopes Specialties, Inc., Burbank, Calif., and had a specific activity of 6.02 mc./*m*M. All C^{14} determinations were performed with a windowless, gas-flow counter on samples plated as infinitely thin layers on nickel planchets 2.5 cm. in diameter. All samples were counted for a sufficient length of time to insure a counting error of 5% or less. Plants were grown from seeds obtained from the Vaughn Seed Company, Chicago, Ill.

Incorporation of 2-C^{14}-Sodium Acetate into Sterol and Triterpene of Flowers and Main Stem.—A flowering section of *S. officinalis* L. was cut with a razor well below the flowering area and all leaves were severed at the base of the petioles. The cut end of the stem was immersed in a small glass vessel containing 1.0 ml. of 2-C^{14}-sodium acetate in water (a total of 1.1×10^8 c. p. m.). During the absorption period, about twenty minutes, the section was illuminated according to Krotkov and Barker (4). Immediately after the acetate solution was absorbed, 1 ml. of distilled water was placed in the container, after which the section was allowed to stand overnight without illumination. The portion immersed in the solution was then rinsed in tap water and the section was allowed to dry at room temperature for two weeks. It was then cut into small pieces and extracted exhaustively with ethanol. The concentrate of the ethanol extracts was fractionated into nonsaponifiable and potassium hydroxide-soluble, as previously outlined (2). Only that portion of the alcohol concentrate soluble in ethyl ether was examined. The plant material after extraction weighed 0.290 Gm. From the nonsaponifiable fraction sterol was precipitated as the digitonide according to Sperry and Webb (6) and the digitonide subsequently converted to free sterol by the pyridine method (7). The C^{14}-content of the crude sterol mixture is shown in Table I. The crude acidic triterpene fraction was chromatographed on Whatman No. 1 paper as previously described (2). The forty-eight-hour overflow was collected as the oleanolic acid fraction and the material at the starting line eluted for the ursolic acid fraction. Yield and C^{14}-content of the crude fractions are shown in Table I also. To the crude sterol fraction, 100.0 mg. of β-sitosterol was added. To the crude overflow material, 100.0 mg. of oleanolic acid, and to the material eluted from the starting line, 100.0 mg. of ursolic acid I was added. Each mixture was then crystallized twice, the β-sitosterol from acetone-methanol, the acids from aqueous or absolute ethanol. Approximately 50 mg. of each sample was at this point taken for acetylation; the remainder was reserved for further crystallization. Acetylation was performed by refluxing each sample one-half hour with 3.0 ml. acetic anhydride and anhydrous pyridine, respectively. The cooled mixture was diluted with water, extracted with ether, and then washed successively with water, 10% hydrochloric acid, water, 5% sodium bicarbonate, and water. The ether was distilled and the product recrystallized, the β-sitosterol acetate from acetone-methanol, the acetylated acids from aqueous ethanol. The free compounds were recrystallized eight times, the acetates four times. There was no change in specific activity of the free compounds or acetates after two crystallizations (Table II). Saponification of the acetates and two crystallizations of the free compound so-obtained gave material with a specific activity in agreement with that obtained by direct crystallization without acetylation.

TABLE II.—INCORPORATION OF 2-C^{14}-SODIUM ACETATE INTO β-SITOSTEROL, URSOLIC ACID I, AND OLEANOLIC ACID OF *S. officinalis* ORGANS

Plant Organ	Specific Activity, c. p. m. per mg.					
	β-Sitosterol		Ursolic Acid		Oleanolic Acid	
	Free	Acetate	Free	Acetate	Free	Acetate
Flowers and stem	184	167	1,177	1,126	53	40
Leaves only	96	84	141	129	192	180

Incorporation of 2-C^{14}-Sodium Acetate into Sterol and Triterpene of Leaves.—Four large leaves from the same plant used for the previous experiment were immersed at the severed end in 1.0 ml. of the 2-C^{14}-sodium acetate solution and otherwise treated as indicated for the flowering stem section. The plant material after ethanol extraction weighed 0.197 Gm. Yield and C^{14}-content of crude sterol and triterpene fractions and respective diluted and recrystallized fractions are shown in Tables I and II.

RESULTS AND DISCUSSION

C^{14} was incorporated from 2-C^{14}-sodium acetate into both sterol and acidic triterpenes of flowers and stem devoid of leaves or into isolated leaves of *S. officinalis* L. (Tables I and II). Because it was necessary to resort to considerable dilution of the various fractions with nonradioactive material in

order to obtain sufficient product for repeated crystallizations, some caution must be observed in interpreting the specific activity figures shown in Table II. It is obvious, however, that only a relatively small proportion of the radioactivity in the crude acidic triterpene fractions was contributed by the triterpenes themselves. The generally higher specific activity of β-sitosterol and ursolic acid I in the flower-stem section is probably a contribution largely from the flower parts themselves, this being a general phenomenon observed in our studies thus far. On this account and perhaps others, it seems best to delay any further interpretation of the interesting high specific activity shown by ursolic acid I in the flower-stem section. The chromatographic procedure for separating oleanolic acid and the ursolic acids does not separate ursolic acids I and II, the latter both remaining at the starting line. Since both of the ursolic acids are present in *S. officinalis* L. (3) this represents somewhat of a problem for tracer work. For the work herein described it seems highly probable that ursolic acid II, possessing a much greater solubility than ursolic acid I, was removed in the crystallizations described and that the specific activity indicated for ursolic acid I is indeed due to this compound.

In a number of as yet unpublished experiments it has been found that 2-C^{14}-acetate or -mevalonate presented to several Labiates in the manner described gave rise to C^{14}-labeled sterol and triterpenes in all parts of the plants examined. Barring actual transport of sterol or triterpene during the experiments (which seems highly unlikely at this time), this information together with the present report gives strong evidence in favor of sterol and triterpene synthesis *in situ* for either leaf, stems, or flowers. It should be added that because of the diverse pathway taken by acetate in its metabolism, the present data are not informative regarding the mode of biosynthesis of either sterol or triterpene.

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Notes

Saccharin Derivatives V. Synthesis of 5-Aminosaccharin and Related Compounds

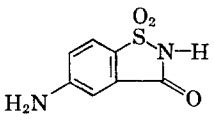
By ALTON WARREN and GLENN H. HAMOR

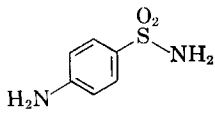
The synthesis of the following compounds is reported: 5-nitrosaccharin, 5-aminosaccharin, 5-acetamidosaccharin, 2-methyl-5-nitrosaccharin, and 2-ethyl-5-nitrosaccharin. These substances lacked a sweet taste, being either bitter or tasteless.

AS PART of a continuing study of saccharin derivatives, the preparation of 5-aminosaccharin and related compounds was desired. Various saccharins have been reported to have local anesthetic (1) or analgesic and antihistaminic (2) activity. The compounds were also synthesized to be included in a forthcoming paper on the relationship of chemical structure to taste in the saccharin series.

The 5-nitrosaccharin was needed to further substantiate previous work in which attempts to prepare 5-nitrosaccharin, using procedures described in the literature, gave instead a mixture of two nitrosaccharins which was shown to consist of 6-nitrosaccharin and 4-nitrosaccharin (3).

Our final reason for synthesis of 5-aminosaccharin will have primarily historical meaning. Backeberg and Marais in the early 1940's were interested in the preparation of 5-aminosaccharin because of its structural similarity to sulfanilamide, as shown below (4). They obtained 5-acetamidosaccharin but could not get the desired compound on deacetylation attempts. With the knowledge that sulfanilamide exerts its bacteriostatic action by competing with *p*-aminobenzoic acid (5), there should be no logical reason for expecting 5-aminosaccharin to have similar activity. However, 5-aminosaccharin was synthesized to fill this void in the history of sulfanilamide-like compounds.





The parent compound, 5-nitrosaccharin, from which all the others were prepared, was synthesized according to the following series of reactions:

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