tion was characterized not by being the best at the early stages of the study, but rather by showing a very slow increase in R as the amount of data was increased. Further studies are planned as to the generality of this behavior.

The unusual features of the phytolaccagenin structure are its high degree of oxidation and the appearance of a carbomethoxy group. Other triterpene esters have been isolated,[5] but their formation has been attributed to esterification during treatment with acidic alcohol. Since phytolaccagenin can be prepared without any exposure to methanol its ester function is apparently of natural origin.

Phytolaccagenin represents one of the largest natural products to be solved without the use of a heavy atom, and its solution exemplifies the growing possibilities for the use of chemical information in the early stages of an X-ray structural analysis.

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RECEIVED DECEMBER 5, 1963

Evidence that Cholesterol Sulfate is a Precursor of Steroid Hormones

Sir:

Since the initial observation[1] that conjugation of steroids does not necessarily destine the hormone for excretion, two important findings have supported the idea that intact steroid sulfates may serve as biosynthetic intermediates. In a recent study, Calvin, *et al.*,[2] found that urinary dehydroisoandrosterone sulfate (DS) may be synthesized *in vivo* from pregnenolone sulfate (PS) by a pathway involving intact steroid sulfates as intermediates. They have also shown[3] that a homogenate of hyperplastic tissue is able to hydroxylate PS at C-17.

To extend these findings, cholesterol-7α-^{3}H sulfate-^{35}S (CS) (24.9×10^6 c.p.s. ^{3}H and 4.35×10^6 c.p.m. ^{35}S, ^{3}H–^{35}S ratio = 5.7) was injected into the left splenic artery of a female subject with an inoperable adrenal carcinoma. This vessel supplied 90% of the blood going to the neoplasm. The CS was injected through a small catheter introduced into the artery under radiographic visualization prior to the selective perfusion of the neoplasm with an antimetabolite.

The conjugates from the first 24-hr. urine collection were extracted into ethanol–ether (3:1).[4] After evaporation of the organic solvents, the remaining aqueous phase was extracted with n-butyl alcohol. The dry residue left after removal of the butyl alcohol contained 2.5% of the injected radioactivity. It was purified by partition chromatography on Celite using the system[5]: isooctane–t-butyl alcohol–1 M NH_4OH (2:5:5), which separates the monosulfates from other urinary conjugates. The sulfate fraction was distributed between 0.3 M pyridinium sulfate solution and chloroform.[6] Evaporation of the $CHCl_3$ afforded the pyridinium salts of the steroid sulfates (264,000 c.p.m. ^{3}H and 40,000 c.p.m. ^{35}S) which were purified further by partition chromatography on Celite using the system: isooctane–chloroform–n-butyl alcohol–methanol–0.3 M pyridinium sulfate–pyridine (4:2:0.7:2:2:0.1). At least two radioactive metabolites were eluted, the more polar of which was rechromatographed on Celite using the system: isooctane–ethyl acetate–n-butyl alcohol–methanol–1 M NH_4OH (2:4:1:2:3). One crystalline radioactive product was eluted in the third to fifth hold-back volume, and this was identified by its infrared spectrum as the ammonium salt of DS. It weighed 87 mg. and contained 92,900 c.p.m. ^{3}H and 17,100 ^{35}S. The product was recrystallized from a mixture of MeOH and acetone to constant specific activity with respect to both ^{3}H and ^{35}S in both crystals and mother liquor residues during three successive crystallizations. The data are presented in Table I, where the ^{3}H–^{35}S ratios are also shown. The latter values were essentially identical with the ratio present in the injected CS. In addition, the specific activities with respect to both isotopes remained constant following conversion of the DS to its oxime and two crystallizations of that derivative from methanol–ether. The yield of radioactivity associated with DS in the urine of the first day was approximately 0.5%.

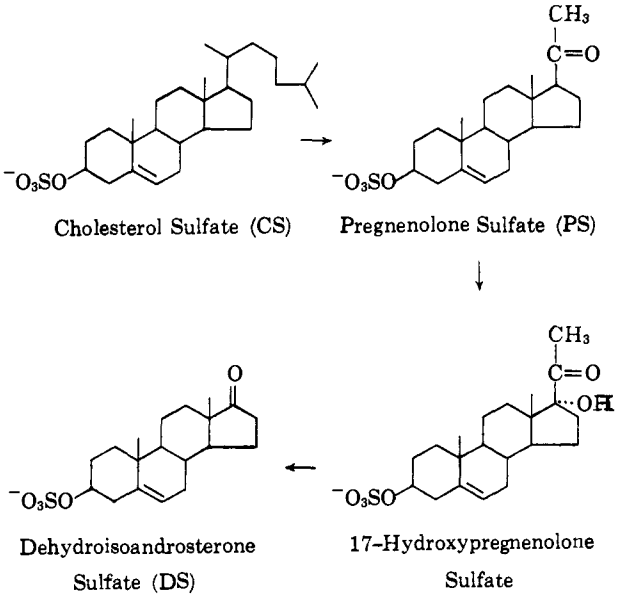
TABLE I

CRYSTALLIZATION DATA

Sample	Specific activity with resp. to ^{3}H in c.p.m./mg.	Specific activity with resp. to ^{35}S in c.p.m./mg.	^{3}H–^{35}S ratio
1st Crystallization	1134	212	5.4
Mother liquor	970	174	5.6
2nd Crystallization	1154	210	5.5
Mother liquor	1090	200	5.5
3rd Crystallization	1141	204	5.6
Mother liquor	1155	213	5.4

SCHEME I

PROPOSED BIOSYNTHETIC PATHWAY INVOLVING STEROID SULFATES AS INTERMEDIATES



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This demonstration of the conversion of CS to DS, a major secretory product of the adrenal,[7] lends further support to the concept that sulfated intermediates, such as CS, PS, etc., are involved in the biosynthesis of the steroid hormones. Although the possibility of other pathways has not been excluded, the evidence available to date suggests that the conversion proceeds *via* C_{21} intermediates as shown in Scheme I.

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Identification of Peroxy Radicals by Electron Paramagnetic Resonance

Sir:

In e.p.r. studies free radicals are identified by their characteristic hyperfine splittings, their g values (line positions), and their relaxation times in a particular milieu. The last parameter is the least specific and the first parameter gives the most information. Peroxy radicals, $RO_2\cdot$, have no hyperfine structure detectable by present methods except that due to O^{17}. Because of the difficulty of O^{17} labeling only one experiment on the O^{17} hyperfine splittings of free radicals has been reported,[1] so that method is not yet broadly applicable.

We wish to report evidence that the g values of peroxy radicals easily distinguish them from $RO\cdot$ and $R\cdot$. We have measured the g value of the cumyl peroxy radical which is 2.0155 ± 0.0005. Lontz[2] found that the radical $CF_3\dot{C}FCONH_2$ reacts in the solid state with molecular oxygen to form a free radical without noticeable hyperfine splitting and with g values measured along the three principal axes of 2.0102, 2.0193, and 2.0267. He assumed this is the corresponding peroxy radical $CF_3\underset{\displaystyle O_2}{\overset{|}{C}}FCONH_2$. Fessenden and Schuler[3] found that alkanes irradiated with fast electrons in the absence of oxygen formed hydrogen atoms plus readily identifiable alkyl radicals. In the presence of oxygen under the same conditions radicals without noticeable hyperfine splitting were produced. The g values of these latter radicals ranged from 2.0155 when pentane was used to 2.0148 for paraffin oil, the g value decreasing with increasing molecular weight of the parent alkane. These were also presumed to be peroxy radicals.

Our cumyl peroxy radicals were produced in benzene solution as a result of the reaction of cumene with oxygen in the presence of azobisisobutyronitrile. No other radicals were detected in this system by e.p.r. Evidence that the principal radical formed in this system is in fact the cumyl peroxy radical, and the experimental arrangement, are discussed by Thomas.[4]

Ordinary free radicals of the type $R\cdot$ or $RO\cdot$[5] (or even $R_2NO\cdot$[1]) all have g values of less than 2.0100. Consequently, the location of the solution g values of peroxy radicals in the range 2.0140 to 2.0190 clearly distinguishes them from these other radicals. One of McConnell and Robertson's generalizations[6] originally applied to aromatic radicals, *i.e.*, the g value of a radical increases with the spin orbit coupling parameter, ζ, of the atoms that have significant spin density, applies also to this case.

To remove some spin density from the terminal oxygen atom and distribute it to any extent on the R group requires the polarization of the O–O bond so as to make the terminal oxygen more negatively charged. For this reason radicals with electron-withdrawing groups in R, such as the radical of Lontz, should have the greatest spin density on the terminal oxygen atom and therefore the highest g value. In solution this radical would have the averaged g value of 2.0187.

Our microwave frequency was measured by a Hewlett–Packard 524C counter with a Hewlett–Packard 540B transfer oscillator. The magnetic field was determined by proton magnetic resonance using a marginal oscillator whose frequency was measured on the same 524C counter. The line broadening effect of the presence of molecular oxygen was a principal source of error in the g value measurement.

(1) J. C. Baird, *J. Chem. Phys.*, **37**, 1879 (1962).

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RECEIVED JANUARY 8, 1964

Studies of Reactions of Atomic Carbon by a Double Tracer Technique

Sir:

Reaction mechanisms of atomic carbon have recently been studied using C^{11} (20.5 min.) as produced by nuclear transformation.[1-4] The C^{11}, being radioactive, serves as its own tracer. However, it would also be greatly desirable to trace the origin of the atoms with which the C^{11} combines. Because the nuclear recoil technique provides only a small number of carbon atoms ($\sim 10^8$) and hence of product molecules, this has not been practical thus far. Labeling the molecules with which the carbon reacts with another radioisotope is not feasible because the amount of such a secondary label which could be incorporated in the product is too small to detect.[5]

The use of stable isotopes, such as deuterium, as secondary tracers has faltered on the formidable problem of product analysis. Mass spectrometric separation of isotopic deuterated molecules followed by counting of their C^{11} content is possible in principle, but very difficult in practice.[6] The alternative, gas chromatographic separation of isotopic molecules followed by C^{11} radioassay, has been restricted by the unavailability of columns of sufficient resolving power.

With the recent publication by Cvetanovic, *et al.*,[7] of a technique for separation of deuterated light olefins

(1) C. MacKay, *et al.*, *J. Am. Chem. Soc.*, **84**, 308 (1962).

(2) C. MacKay and R. Wolfgang, *ibid.*, **83**, 2399 (1961).

(3) G. Stöcklin and A. P. Wolf, *ibid.*, **85**, 229 (1963).

(4) C. MacKay and R. Wolfgang, *Radiochim. Acta*, **1**, 42 (1962).

(5) In a typical experiment 10^8 carbon atoms might react with a hydrocarbon labeled with tritium. Even if the hydrocarbon were completely labeled this would result in only an order of 10 to 100 tritium disintegrations per min. in the 10^8 product molecules. Furthermore with a totally labeled molecule tritium self-radiolysis would be a serious problem.

(6) Mass spectrometric separation of trace isotopic product molecules would require high throughput efficiencies. Furthermore special counting techniques for the separated C^{11} labeled molecules would have to be developed.

(7) R. J. Cvetanovic, F. J. Duncan, and W. E. Falconer, *Can. J. Chem.*, **41**, 2095 (1963).