Preparation of Sulfate Esters. Reactions of Various Alcohols, Phenols, Amines, Mercaptans, and Oximes with Sulfuric Acid and Dicyclohexylcarbodiimide[1]

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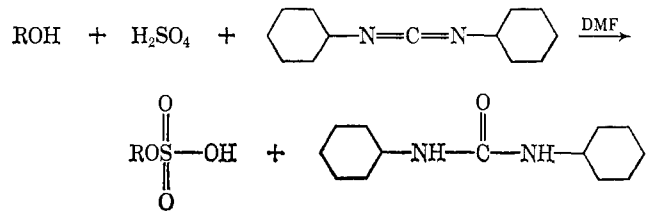
Abstract: The versatility of a dicyclohexylcarbodiimide-mediated sulfation reaction[2] was examined. Various alcohols, phenols, mercaptans, amines, oximes, and other biologically related molecules were added to radioactive sulfuric acid and dicyclohexylcarbodiimide (DCC) in the solvent dimethylformamide (DMF). All the nucleophiles examined were observed to form sulfate esters. Reaction conditions were found which selectively sulfated alkyl hydroxyl groups in good yields but did not sulfate other nucleophiles such as phenols, mercaptans, oximes, or amines. This selective hydroxyl sulfation was utilized in the synthesis of a number of biologically related molecules, *e.g.*, *estradiol 17β-sulfate.*

Sulfate esters have long been chemically important in the detergent and dye industries, as well as in the production of alcohols from alkenes. Recently, biochemists have become quite interested in sulfation because of the great number of naturally occurring sulfates which have been found in both plant and animal tissues. Some of the classes of sulfates which are synthesized in biological systems are the alkyl, aryl, steroidal, and carbohydrate sulfates.[3] Other chemically related compounds which are of biological importance are the thiosulfates ($R-S-SO_3H$) and the sulfamates (R_2-NSO_3H). The thiosulfate compounds (Bunte salts) have been postulated as intermediates of sulfate reduction.[4]

Two reactions have been commonly used for the preparation of sulfate esters: (1) the reaction of sulfuric acid with an alkene, or (2) the reaction of sulfur trioxide or a derivative thereof with an alcohol. In this second class of sulfation reactions, numerous reagents have been employed such as sulfur trioxide, chlorosulfonic acid, fuming sulfuric acid, sulfamic acid, and pyridine–SO_3 adducts.[5]

In 1966, Mumma reported the synthesis of monosulfate esters by a third method, a dicyclohexylcarbodiimide (DCC) mediated sulfation of alcohols.[2] The monosulfate structure of the products was verified by elemental, infrared, and melting point analyses. Recent laser ionization mass spectrometry has also confirmed the structure of these DCC-synthesized monosulfates.[6]



In our present investigation, the versatility of the DCC-mediated sulfation reaction was examined. Various nucleophiles such as alcohols, phenols, mercaptans, amines, and oximes were added to radioactive sulfuric acid and DCC in the solvent dimethylformamide (DMF) at a selected temperature, molar ratio, and reaction time. Reaction conditions were examined to determine whether a selective sulfation of specific nucleophiles is possible, such that the experimental conditions would allow the selective sulfation of polyfunctional molecules. The advantages of this DCC-mediated sulfation reaction would be that it would simplify previously used methods of synthesis and also provide milder reaction conditions for the preparation of biologically related compounds containing ^{35}S-labeled sulfate groups.

Experimental Section

Nucleophiles. Various nucleophiles, whose functional groups are similar to those found on biological molecules, were subjected to the DCC-mediated sulfating conditions. Of these nucleophiles, the alcohols, phenols, mercaptans, amines, sugars, a nucleoside, and steroids were purchased. The cyclohexanone oxime,[7] acetophenone oxime,[8] and benzenethiol[9] were synthesized. Most of the alcohols, phenols, mercaptans, amines, and all the DMF (Matheson Coleman and Bell, industrial grade) were distilled in an all-glass apparatus and stored under refrigeration until used.

The purity of all the reactants was determined by gas chromatography (glpc) (Barber Colman 5000 gas chromatograph), using a 3% OV-1 (dimethyl silicone) column at three temperatures (130, 160, and 200°). The purity of the nucleophiles was also analyzed by thin layer chromatography (tlc), coupled with selective sprays, and by their melting and boiling points.

Sulfuric Acid. Carrier-free $H_2{}^{35}SO_4$ was obtained from New England Nuclear Corporation (Boston, Mass.). The purity of the $^{35}SO_4{}^{2-}$ was checked by paper electrophoresis at pH's 2, 7, and 11.

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(7) J. Cason and H. Rapoport, "Basic Experimental Organic Chemistry," Prentice-Hall, Inc., Englewood Cliffs, N. J., 1965, p 121.

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Approximately 5 mCi of $^{35}SO_4^{2-}$ was placed in separate vials and neutralized with excess ammonium hydroxide. The vials were then air blown to dryness. To each vial, 1 ml of concentrated H_2SO_4 was added to give an activity of 5 mCi of ^{35}S per 1 ml of H_2SO_4.

Carbodiimides. Although many types of carbodiimides are available, dicyclohexylcarbodiimide (DCC) (Eastman Organic Chemicals) was selected because of the stability of DCC and because of its wide use in phosphate chemistry.[10-12]

Reaction Conditions. The nucleophiles (N), DCC, and H_2SO_4 were allowed to react in fixed molar ratios of approximately 1:5:1, respectively, in the solvent DMF at two concentrations here designated as "dilute" and "concentrated."

"dilute"	0.24 mmol of N/3.00 ml of DMF	
	1.15 mmol of DCC/4.00 ml of DMF	(10 ml)
	0.24 mmol of H_2SO_4/3.00 ml of DMF	
"concentrated"	0.24 mmol of N/0.30 ml of DMF	
	1.15 mmol of DCC/0.40 ml of DMF	(1 ml)
	0.24 mmol of H_2SO_4/0.30 ml of DMF	

Two reaction controls were run with each nucleophile. The control reaction for the "dilute" conditions was conducted by adding 0.24 mmol of H_2SO_4 (25 mg of concentrated H_2SO_4, 95%), dissolved in 3 ml of DMF, to 0.24 mmol of nucleophile, dissolved in 7 ml of DMF. The control reaction of the "concentrated" conditions was prepared by adding 0.24 mmol of H_2SO_4, dissolved in 0.3 ml of DMF, to 0.24 mmol of nucleophile, dissolved in 0.7 ml of DMF.

The reaction sequence followed was to place a vial containing the DCC–DMF solution in an ice bath. To this was added the nucleophile, which had previously been dissolved in DMF. Then the specified amount of precooled DMF was added to the vial containing the $H_2{}^{35}SO_4$. The DMF–H_2SO_4 solution was then shaken and immediately transferred by pasteur pipet to the DCC–nucleophile–DMF mixture. If the H_2SO_4–DMF solution was mixed and allowed to stand for more than 1 hr before use, undesirable ^{35}S side products were observed. After all the components had been combined, the mixture was occasionally shaken for 15 min, and then immediately spotted on thin layer and paper chromatograms.

Chromatography. Thin layer chromatography (tlc) was performed with 0.3 mm thick silica gel (Supercosil-12B, Supelco Inc.) plates. Three different solvent systems were used for developing the plates as follows: (1) chloroform–methanol–water (65:25:4, v/v); (2) chloroform–methanol–water (65:40:8, v/v); and (3) benzene–diethyl ether–ethanol–acetic acid (50:40:2:0.2, v/v).[13] Linerless tanks were allowed to equilibrate with developing solvents for 1 hr before use. Plates were stained with iodine, charred with 20% perchloric acid, and examined with various specific sprays. Phenols were detected with diazotized sulfanilic acid[14] and potassium permanganate[15] sprays. A *p*-toluenesulfonic acid spray was used for steroids, sodium nitroprusside spray for mercaptans, potassium ferricyanide–ferric chloride spray for thiosulfates, and ninhydrin and sodium nitroprusside (specific for secondary aliphatic and alicyclic amines) sprays for amines.[15]

One-dimensional paper chromatography (pc) was carried out using Whatman No. 4 filter paper. Phenol–water (100:40, w/w) was used as a descending developing system.

Kodak single-coated, blue-sensitive X-ray film was placed on all radioautograms for at least 24 hr in X-ray exposure holders. After the proper amount of exposure, the films were developed in General Electric Supermix X-ray Developer and Fixer.

Determination of the Yields of ^{35}S-Labeled Products. The yield for each ^{35}S-labeled product was determined by tlc and/or pc as the ratio of ^{35}S-labeled products (cpm) to the total amount of ^{35}S (cpm) present on the chromatogram, expressed as a percentage. The chromatograms were sectioned and counted in a Tri-Carb liquid scintillation spectrometer (Model 526 Packard Instrument Co.). A yield of less than 0.5% was considered to be zero.

Hydrolysis of the ^{35}S-Labeled Products. The radioactive labeled products were eluted from paper chromatograms by chloroform–methanol–water (6:3:1, v/v). These eluted ^{35}S-labeled products were then hydrolyzed in 1 *N* aqueous HCl for 1 hr in a steam bath and then paper chromatographed. Products which failed to hydrolyze in 1 hr under these conditions, as evidenced from chromatographic analysis, were reeluted and rehydrolyzed for 24 hr. The eluted paper chromatograms were checked with X-ray film for complete removal of the ^{35}S-labeled compounds.

Results

Sulfation of Alcohols. Under the previously stated conditions, various primary and secondary alcohols were sulfated in good yields. The alcohols sulfated were ethanol, 1-butanol, 1-hexanol, 1-octanol, 1-decanol, 1-dodecanol, 1-tetradecanol, 1-hexadecanol, 1-octadecanol, 2-propanol, 2-butanol, and cyclohexanol. A tertiary alcohol, 2-methyl-2-propanol, also yielded a ^{35}S-labeled product, but it was observed only by tlc. Its paper chromatogram, which was developed in phenol–water, showed no radiochemical evidence of ^{35}S-labeled product formation. This may be accounted for by the nonstability of tertiary sulfates at room temperatures.[16-18]

Under the "dilute" conditions for all alcohols, only one product, the monosulfate, was observed. The proof of the monosulfate structure was based on previous elemental, melting point, and infrared analyses.[2] Additional evidence was obtained in the present investigation by 1-hr hydrolysis of the ^{35}S-labeled products and by laser ionization mass spectrometry.[6]

Further confirmation of the monosulfate structure was obtained by thin layer cochromatography of the 1-tetradecyl sulfate synthesized by the DCC-mediated reaction with purchased 1-tetradecyl sulfate (Mann Research Lab., Inc., New York, N. Y.) and also with 1-tetradecyl sulfate synthesized by a pyridine–$^{35}SO_3$ sulfation of 1-tetradecanol.[5] The R_f's observed in the thin layer cochromatography were as follows: 0.65 with the solvent system chloroform–methanol–water, 65:25:4, v/v (CMW); 0.63 with chloroform–methanol–water, 65:30:6, v/v; 0.90 with chloroform–methanol–water–pyridine, 65:50:8:0.5, v/v; 0.83 with 1-propanol–NH_4OH–water, 60:30:10, v/v; 0.73 with 1-butanol–pyridine–water, 3:2:1.5, v/v.

Under the "concentrated" conditions two ^{35}S-labeled products were observed, one of which cochromatographed with the monosulfates, while the second ran with the solvent front. This additional product appears to be a pyrosulfate diester ($ROSO_2OSO_2OR$), analogous to the sulfonic anhydrides reported by Khorana.[11] This conclusion was supported by the evidence that when this front-running compound was eluted and rechromatographed, partial decomposition occurred, with the formation of monosulfate and no detectable amounts of $H_2{}^{35}SO_4$. Products such as $ROSO_2OSO_2OH$ can therefore be excluded.

DCC was shown to be an absolute requirement for sulfation under "dilute" and "concentrated" conditions, since ^{35}S-labeled products were not formed in the control reactions (nucleophile and H_2SO_4 in DMF). The yields of four alcohol sulfates were radiochemically computed (Table I). A radioautogram drawing of the 1-octanol experiment is shown in Figure 1 and this representation is typical of all the experiments.

(10) H. G. Khorana, *Chem. Rev.*, **53**, 145 (1953).
(11) H. G. Khorana, *Can. J. Chem.*, **31**, 585 (1953).
(12) H. G. Khorana, "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest," John Wiley & Sons, Inc., New York, N. Y., 1961, Chapter 6.
(13) C. P. Freeman and D. West, *J. Lipid Res.*, 7, 324 (1966).
(14) J. G. Krishna, H. W. Dorough, and J. E. Casida, *J. Agr. Food Chem.*, **10**, 463 (1962).
(15) D. Waldi in "Thin-Layer Chromatography," E. Stahl, Ed., Academic Press, New York, N. Y., 1965, pp 485–502.
(16) W. H. Brader, *Chem. Abstr.*, **59**, 8595 (1963).
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(18) L. F. Fieser, *ibid.*, **70**, 3232 (1948).

Table I. Yield Data for the DCC-Mediated Sulfation of Alcohols

Compound	Concentrations	ROH, $H_2{}^{35}SO_4$, and DCC Tlc	Pc
1-Octanol	Dilute	1 (78)[a]	1 (77)
	Concentrated	2 (100)	2 (99)
Cyclohexanol	Dilute	1 (45)	1 (42)
	Concentrated	2 (95)	2 (96)
1-Tetradecanol	Dilute	1 (80)	*b*
	Concentrated	2 (95)	
Cholesterol	Dilute	1 (45)	*b*

[a] The total number of ^{35}S-labeled products observed and the combined percentage yield (%). [b] Experiment not conducted.

Sulfation of Phenolic Compounds. Phenol, α-naphthol, and β-naphthol did not form sulfate esters under "dilute" conditions (Table II). However, under the "concentrated" conditions, two products were observed. One of these products cochromatographed with the monosulfate, while the other ran as a neutral species with the solvent front on both thin layer and paper chromatograms. This front-running product is presumably a pyrosulfate diester, since mild acid hydrolysis converts it to the monosulfate. All ^{35}S-labeled products were found to be hydrolyzed in 1 hr to the starting phenolic compound (tlc sprays and glpc) and H_2SO_4.

Table II. Yield Data for the DCC-Mediated Sulfation of Phenolic Compounds

Compound	Concentrations	ROH, H_2SO_4, and DCC Tlc	Pc
Phenol	Dilute	1 (<1)[a]	0 (0)
	Concentrated	2 (95)	2 (93)[b]
α-Naphthol	Dilute	0	0 (0)
	Concentrated	2	2 (99)[b]
β-Naphthol	Dilute	0	0 (0)
	Concentrated	2	2 (99)[b]
Benzene	Dilute	0 (0)	0
	Concentrated	0 (0)	0
Naphthalene	Dilute	0 (0)	0
	Concentrated	0 (0)	0

[a] The total number of ^{35}S-labeled products observed and their combined percentage yield (%). [b] Product(s) completely hydrolyzed in 1 hr.

As additional controls, benzene and naphthalene were subjected to the same sulfation conditions. As expected, these aromatic hydrocarbons did not form ^{35}S-labeled products.

Under the "dilute" conditions, the lack of formation of phenolic monosulfate esters may be explained by the resonance of the electrons of the oxygen atom with the aromatic ring, making the phenolic hydroxyl a weaker nucleophile than an alcohol. Potassium permanganate spray was used for the detection of the ^{35}S-labeled products. The unreacted phenolic compounds turned yellow, while the ^{35}S-labeled products produced a different shade of yellow 20 min later.

Reactions with Mercaptans. Various aliphatic and aromatic mercaptans, such as 1-octanethiol, 1-dodecanethiol, benzenethiol, 2-naphthalenethiol, and α-toluenethiol, were examined. Under "dilute" conditions essentially no ^{35}S-labeled products were formed; however,

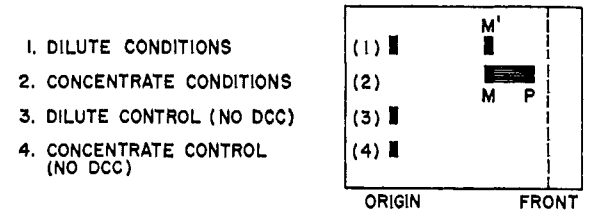


Figure 1. Radioautogram drawing of the DCC-mediated sulfation experiment with 1-octanol. The paper chromatogram was developed in phenol–water (100:40 w/w). The yields were M′ = 77%; M = 55%; and P = 45%.

under the "concentrated" conditions two ^{35}S-labeled products were obtained. One product corresponds to the thiosulfate ester (Bunte salt) and the other to presumably the thiosulfate ester anhydride ($RSSO_2OSO_2SR$), which migrates to the front during chromatography (Table III). The low yields for benzenethiol and 2-naphthalenethiol under "concentrated" conditions may possibly be explained by the resonance of the electrons of the sulfur atoms with the aromatic rings, thus weakening the nucleophilic strength of the mercaptans. Moreover, a higher product yield is observed with α-toluenethiol ($C_6H_5CH_2SH$), where resonance of electrons of the sulfur with the aromatic ring is prevented by a methylene group.

Table III. Yield Data for the DCC-Mediated Sulfation of Mercaptans

Compound	Concentrations	RSH, H_2SO_4, and DCC Tlc	Pc
1-Octanethiol	Dilute	1 (1)[a]	1 (1)
	Concentrated	2 (100)	2 (92)[d]
1-Dodecanethiol	Dilute	0	0 (0)
	Concentrated	2	2 (80)[d]
Benzenethiol	Dilute	0 (0)	0
	Concentrated	2 (50)	1[b]
2-Naphthalenethiol	Dilute	0	0 (0)
	Concentrated	2	2 (7)[c]
α-Toluenethiol	Dilute	0 (0)	0 (0)
	Concentrated	2 (80)	2 (90)[d]

[a] The total number of the ^{35}S-labeled products observed and their combined percentage yield (%). [b] Product(s) completely hydrolyzed in 1 hr. [c] Product(s) 50% hydrolyzed in 1 hr. [d] Product(s) hydrolyzed in 24 hr.

The sodium nitroprusside spray visualized the mercaptans as a pink color, while the products were unaffected. A thiosulfate spray stained the ^{35}S-labeled products blue, and the mercaptans brown. These data suggest that the products formed are thiosulfates. This structure has been confirmed by elemental and laser ionization mass spectrometric analysis,[6] and will be presented at a later date. The ^{35}S-labeled products appeared to be more stable to acid hydrolysis than the alcohol monosulfates, since they required a longer time of hydrolysis. Because of their high volatility at 100°, the mercaptans, formed by hydrolysis of the products, could not be visualized by tlc sprays, but was suggested by the formation of their characteristic odors.

Reactions with Amines. The primary, secondary, and tertiary amines examined were *n*-propylamine, di-*n*-

propylamine, cyclohexylamine, aniline, and N,N-dimethylaniline. The number of products formed and the yields computed are shown in Table IV. Under the "dilute" conditions, aniline was found to be the only amine to form an ^{35}S-labeled product, presumably a sulfamate. Under "concentrated" conditions all the amines formed two or three products. Although an ^{35}S-labeled product might not be expected with N,N-dimethylaniline, products were observed under "concentrated" conditions. These products may possibly be explained by the known stability of quaternary ammonium sulfamates.[19]

Table IV. Yield Data for the DCC-Mediated Sulfation of Amines

Compound	Concentrations	RNH_2, H_2SO_4, and DCC Tlc	Pc
n-Propylamine	Dilute	0 (0)[a]	0
	Concentrated	2 (20)	0
Di-*n*-propylamine	Dilute	0 (0)	0
	Concentrated	3 (20)	0
Cyclohexylamine	Dilute	0 (0)	0
	Concentrated	3 (33)	0
Aniline	Dilute	1 (7)	1 (7)[b]
	Concentrated	3 (61)	2 (60)[b]
N,N-Dimethylaniline	Dilute	0 (0)	0
	Concentrated	3 (10)	0

[a] The total number of ^{35}S-labeled products observed and their combined percentage yield (%). [b] Product(s) completely hydrolyzed in 1 hr.

The ninhydrin spray (except with N,N-dimethylaniline) and the I_2 staining proved useful in visualizing the amines. However, none of the sprays prepared would visualize the ^{35}S-labeled products, demonstrating the lack of typical amino properties. The products which were observed on tlc, except those of aniline, were not detected on paper chromatograms. Apparently, the phenol–water developing system decomposed the products. Since a protonated amine ($R-NH_3^+$) is a weak nucleophile, the basicity of the amine becomes an important factor. The weak basicity of aniline may account for its greater sulfation reactivity since aniline is least likely to form the protonated amine or compete with proton activation of the DCC. The proof of structure of these sulfamates requires further study.

Sulfation of Oximes. Acetophenone and cyclohexanone oximes were not sulfated under "dilute" conditions. However, under "concentrated" conditions, several ^{35}S-labeled products were observed (Table V). The proof of the structures of these ^{35}S-labeled products will also await further study, but presumably they are sulfated oximes. Oxime derivatives have been found in great abundance in plant tissues.[20] The ^{35}S-labeled products of cyclohexanone oxime partially decompose upon rechromatography. The ^{35}S-labeled products of acetophenone oxime did not decompose during rechromatography, but were hydrolyzed with 1 *N* HCl in 1 hr. Iodine stain visualized both the oximes and their products.

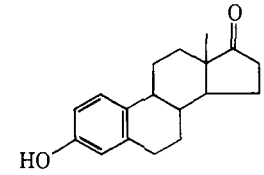
Table V. Yield Data for the DCC-Mediated Sulfation of Oximes

Compound	Concentrations	N, H_2SO_4, and DCC Tlc	Pc
Acetophenone oxime	Dilute	0	0 (0)[a]
	Concentrated	3	2 (45)
Cyclohexanone oxime	Dilute	0	0 (0)
	Concentrated	3	2 (60)

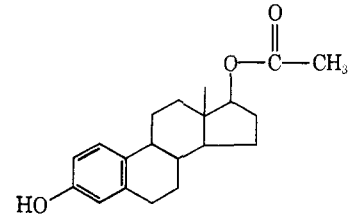
[a] The total number of ^{35}S-labeled products and their combined percentage yield (%).

Sulfation of Biologically Related Molecules. In the preceding experiments, a sulfation selectivity was observed under "dilute" conditions in which hydroxyl groups were easily sulfated while phenolic, mercaptan, and amino groups were not sulfated. Since the most commonly used chemical sulfation procedures require the addition and the removal of blocking groups for the selective hydroxyl sulfation of polyfunctional molecules, this new DCC sulfation reaction will eliminate these time-consuming steps. Also, the quantity of starting material and the number of purification steps are reduced.

Steroids provide an excellent test of this sulfation selectivity, because many steroids have both hydroxyl and phenolic groups. In the attempted sulfations of estrone and estradiol 17β-acetate, no ^{35}S-labeled products (<1%) were observed under "dilute" condition. However, when β-estradiol and β-estradiol 3-benzoate were allowed to react under the same conditions, each formed one ^{35}S-labeled product. This indicated the monosulfation of the steroid's C-17 β-secondary hydroxyl group, since phenolic groups were not sulfated under the "dilute" conditions. The yield of ^{35}S-labeled β-estradiol was 28%, but the amount of product was found to be increased to 65% when the usual molar concentration of H_2SO_4 (0.24 mmol) was doubled. This suggests that further reactant concentration studies are necessary if yields are to be maximized. Other steroids which reacted under the "dilute" sulfation conditions and formed only one ^{35}S-labeled product

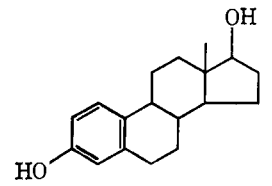


estrone
(1,3,5(10)-estratrien-3-ol-17-one)

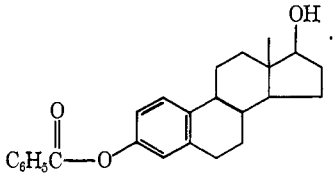


estradiol 17β-acetate
(1,3,5(10)-estratrien-3-ol-17β-yl acetate)

(19) H. H. Sisler and L. F. Audrieth, *Inorg. Syn.*, 2, 173 (1946).
(20) F. Challenger, "Aspects of Organic Chemistry of Sulfur," Butterworth & Co. (Publishers), Ltd., London, 1959, p 115.



β-estradiol
(1,3,5(10)-estratrien-3,17β-diol)



β-estradiol-3-benzoate
(1,3,5(10)-estratrien-17β-ol-3-yl benzoate)

are cholesterol (cholest-5-en-3β-ol), dehydroepiandrosterone (3β-hydroxyandrost-5-en-17-one), lanosterol (cholesta-8,24-diene-4,4,14α-trimethyl-3β-ol), testosterone (17β-hydroxy-13α-14β-androst-4-en-3-one), and deoxycorticosterone (21-dehydroxypregn-4-ene-3,20-dione).

A further selectivity was noted in the sulfation of a molecule possessing a sterically hindered hydroxyl group. Corticosterone (11β,21-dihydroxypregn-4-ene-3,20-dione) formed only one ^{35}S-labeled product, the 21-sulfate. Therefore, it is assumed that the sterically hindered 11β-secondary hydroxyl group cannot be readily sulfated under the "dilute" conditions.

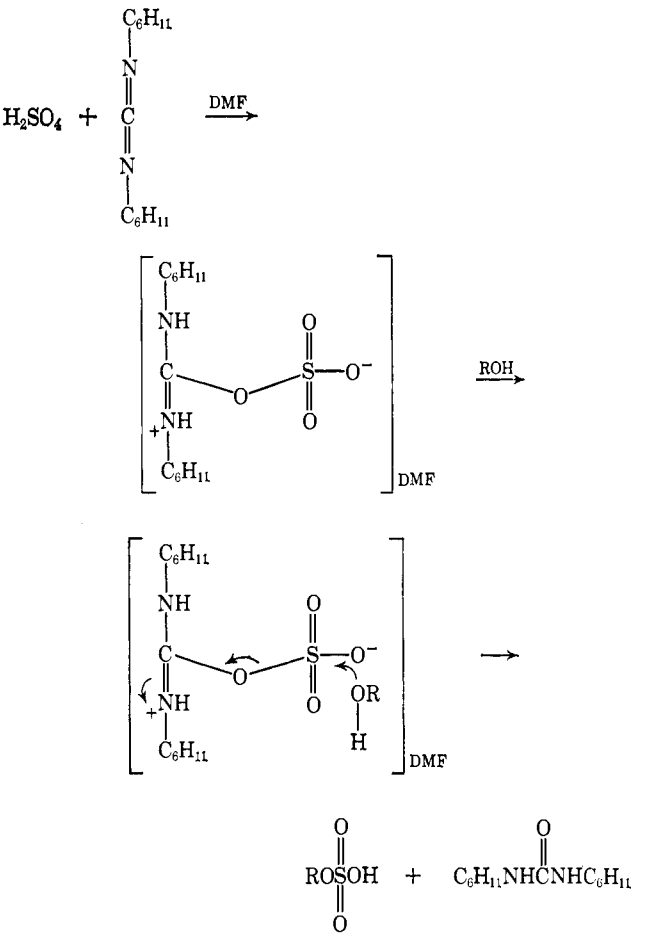
The sulfation under the "dilute" conditions of three derivatives of biological compounds, 2′,3′-isopropylideneadenosine, 1,2:5,6-di-O-isopropylidene-D-glucofuranose, and 1,2:3,4-di-O-isopropylidene-D-galactopyranose, was examined. The cyclic acetal groups were necessary to prevent sulfation of competing hydroxyl groups. All three compounds were observed on both tlc and pc to form one ^{35}S-labeled product. The yields, as determined from tlc, were 25% for 2′,3′-isopropylideneadenosine, 88% for 1,2:5,6-di-O-isopropylidene-D-glucofuranose, and 93% for 1,2:3,4-di-O-isopropylidene-D-galactopyranose.

Discussion

The DCC-mediated sulfation reaction was examined in order to determine whether various nucleophiles could be sulfated. Several reaction conditions were held constant to simplify the interpretation of the results by reducing the number of variables. These constants, such as molar ratio, reaction time, and temperature, were selected in order to favor the formation of the monosulfate. Hence, equimolar ratios of nucleophile to H_2SO_4, the temperature of 4°, and the reaction time of 15 min were selected. Although the reaction occurs rapidly, as evidenced by the immediate precipitation of dicyclohexylurea and the formation of monosulfates (shown by tlc), the reaction time of 15 min was allowed for two reasons: first, compounds of various nucleophilic strengths were to be tested; secondly, completeness of reaction was desired for yield determinations. Since only the degree of reactivity of the various nucleophiles was being examined, the yields were determined radiochemically from the chromatograms of the reaction mixture rather than from isolated products. The reaction conditions were chosen in order to obtain standard conditions for comparison; they are not necessarily optimum for the synthesis of sulfate esters.

Under the reaction conditions DCC was shown to mediate the sulfation of various nucleophiles. The concentration of the reactants in DMF proved to be very important because reaction conditions were found that selectively sulfated alcohols. When the final concentrations of the nucleophile and sulfuric acid were 0.024 *M* in DMF ("dilute") only unhindered hydroxyl groups were sulfated; hindered hydroxyl groups, phenols, mercaptans, amines, and oximes were not sulfated. When the final concentrations of the nucleophile and sulfuric acid were each 0.24 *M* in DMF ("concentrated") all the nucleophiles reacted to form sulfated products. Only one product, the monsulfate ester, was obtained under the "dilute" conditions with the 24 alcohols tested. Under the "concentrated" reaction conditions, two products are formed, the monosulfate ester and presumably the pyrosulfate diester. The products obtained with the amines were unstable and further work is required to establish their structures.

The order of addition of the reactants was found to be important for the formation of ^{35}S-labeled products. The procedure selected was to add the reactants in the following order: (1) DCC, (2) nucleophile, (3) H_2SO_4. The addition sequence of (1) H_2SO_4, (2) nucleophile, (3) DCC was not followed to avoid possible acidic decomposition of the nucleophile before the DCC was added. However, if the previously dissolved components were added in the sequence (1) DCC, (2) H_2SO_4, (3) nucleophile, no ^{35}S-labeled products were observed on thin layer chromatograms, unless the nucleophile was added



to the DCC–H_2SO_4 mixture within 30 sec after the DCC and H_2SO_4 were combined, under the "dilute" conditions. The DCC and H_2SO_4 react in the DMF to form dicyclohexylurea. With the disappearance of DCC, the reaction mixture can no longer sulfate a nucleophile. Therefore, DCC, H_2SO_4, and DMF do not react to form a stable sulfating agent under the "dilute" conditions, such as DMF–SO_3,[21] which would not lose its sulfating ability in 30 sec. Thus the nucleophile appears to be reacting with a DCC–sulfuric acid complex, rather than with a product produced by the dehydration of H_2SO_4, such as sulfur trioxide. Since solvation is one of the most important factors controlling nucleophilicity,[22] DMF may play an active role in the mechanism of the reaction under the "dilute" conditions, when nucleophilic selectivity is found. A reasonable mechanism would involve the formation of a solvolyzed protonated DCC–H_2SO_4 intermediate, followed by a hydroxyl nucleophilic attack to produce a monosulfate ester and dicyclohexylurea.

(21) M. L. Wolfrom and T. M. Shen Han, *J. Am. Chem. Soc.*, **81**, 1764 (1959).
(22) R. F. Hudson, *Chimia*, **16**, 173 (1962).

Analogous intermediates have been postulated[10] for reactions of phosphoric acids and of sulfonic acids (RSO_3H) with DCC under similar reaction conditions. It is reasoned that a protonated adduct enhances the subsequent nucleophilic attack, for it weakens the high π-electron density of the oxygens surrounding the sulfur atom. This hydroxyl attack is further promoted by a slight positive charge residing on the sulfur atom owing to the sulfur–oxygen semipolar bonds of the adduct.

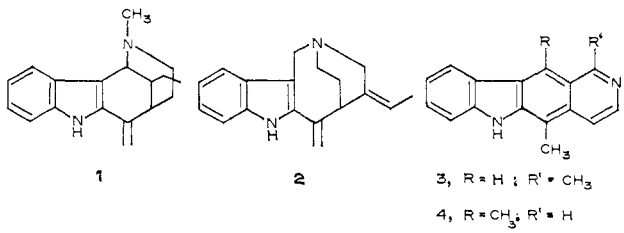
The results of these experiments raise a number of questions which are currently under investigation in our laboratory. The ratio of the reactants used was chosen for comparison purposes; therefore, additional studies are necessary to determine the ratio of reactants for optimal yields of monosulfates. DCC was chosen because of its common use and its availability; however, other carbodiimides might give better yields and perhaps a different selectivity. The techniques of the isolation of gram quantities of the ^{35}S-labeled products are being developed. Additional studies are necessary to determine what role DMF may play in the mechanism and to determine whether solvents other than DMF will also show a selectivity in sulfation.

Communications to the Editor

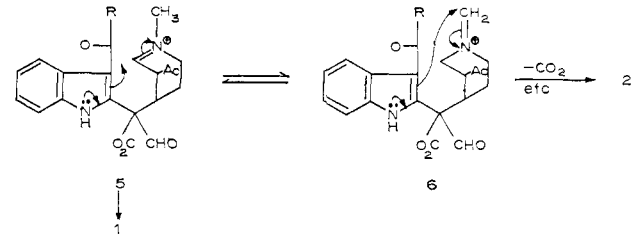
Studies on Indole Alkaloid Biosynthesis. III[1]

Sir:

In previous communications[1,2] we have reported results relating to the later stages of the biosynthesis of Aspidosperma and Iboga alkaloids. Another highly interesting family of indole alkaloids is that which, in common with other Aspidosperma, Iboga, and Corynanthe alkaloids, possesses the ubiquitous C_{10} "non-tryptophan" unit, yet does *not* possess an obvious tryptophan portion. Alkaloids in this family include uleine (**1**)[3] and apparicine (**2**),[4] in which only one carbon atom separates the indole nucleus and the basic nitrogen atom, as opposed to the normal two-carbon bridge of tryptamine and most indole alkaloids. Olivacine (**3**)[5] and ellipticine (**4**),[6] on the other hand, have three carbon atoms between the indole and the nitrogen functions.



No data are presently available on the biosynthesis of these interesting alkaloids. Wenkert[7] has proposed that a progenitor of tryptophan, rather than tryptophan itself, condenses with the C_{10} unit to give **5**, from which uleine, olivacine, and ellipticine could be derived. Djerassi and coworkers,[4] in reporting the structure of apparicine, postulated that a prototropic rearrangement, followed by appropriate cyclization, etc. (**5** → **6** → **2**), would yield the apparicine system. These postulates imply (1) the same precursor for both apparicine and uleine and (2) neither the methylene bridge of apparicine nor the N-methyl of uleine is derived from the tryptophan side chain.



In the hope of obtaining some biosynthetic data relating to these interesting aspects, we have initiated some studies on *Aspidosperma pyricollum*, a plant which has been reported to contain both alkaloids.[8,9]

(1) Part II: J. P. Kutney, C. Ehret, V. R. Nelson, and D. C. Wigfield, *J. Am. Chem. Soc.*, **90**, 5929 (1968).
(2) J. P. Kutney, W. J. Cretney, J. R. Hadfield, E. S. Hall, V. R. Nelson, and D. C. Wigfield, *ibid.*, **90**, 3566 (1968).
(3) G. Buchi and E. W. Warnhoff, *ibid.*, **81**, 4433 (1959).
(4) J. A. Joule, H. Monteiro, L. J. Durham, B. Gilbert, and C. Djerassi, *J. Chem. Soc.*, 4773 (1965).
(5) (a) G. B. Marini-Bettolo and J. Schmutz, *Helv. Chim. Acta*, **42**, 2146 (1959); (b) J. Schmutz and H. Wittmer, *ibid.*, **43**, 793 (1960); (c) E. Wenkert and K. G. Dave, *J. Am. Chem. Soc.*, **84**, 94 (1962).
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(7) E. Wenkert, *ibid.*, **84**, 98 (1962).
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(9) R. R. Arndt and C. Djerassi, *ibid.*, **21**, 566 (1965).