Several cuttings were removed from a plant grown in 50% D_2O nutrient for 40 days. One cutting was transplanted into a culture flask containing 50% D_2O nutrient solution, and the other was placed in a flask containing aqueous nutrient and no D_2O. The former cutting showed no sign of developing a root system and succumbed within 3 to 4 weeks. The latter grew a root system within 2 weeks and continued growing in a normal manner similar to a typical control plant. It was hoped that if a root system would develop in the 50% D_2O medium, it would be possible to propagate a deuteriated plant; and possibly with successive cuttings in constantly increasing concentrations of D_2O a plant could be grown in a highly deuteriated medium. Thus far, this has not been successful, but further work is in progress with this aspect of the investigation.

In Fig. 4 comparison is made between the deuterium content of the water distilled from the deep frozen fresh plants and the water of combustion of the dried plants. The distilled or labile water contains deuterium from water loosely bound by the plant tissues and represents essentially completely exchangeable deuterium. The deuterium content of the labile water from the plant is approximately 75% that of the nutrient solution in which the plant was grown. The water obtained from combustion of the dried plants, representing organically bound exchangeable and nonexchangeable deuterium, contains about 50% of the deuterium content of the nutrient solution. The ratio of organically bound to labile deuterium is about 0.66, somewhat higher than that reported by Katz, *et al.* (6), for certain tissues in mice. This may reflect the nonequilibrium aspects of the situation resulting from the continuous transpiration of water. The active water metabolism and the ensuing fractionation may contribute significantly to the observed isotope effects.

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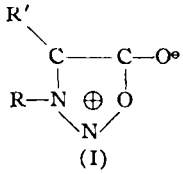
Synthesis of Several Disubstituted Sydnones

By DEVINDRA DHAWAN and LEMONT B. KIER*

A number of new 3,4-dialkylsydnones have been prepared to compare their pharmacological properties with those of a series of 3-alkylsydnones. Both series were similar in their central convulsive activity. The CD_{50} and partition coefficient values of both series were compared. The CD_{50} values for the 3,4-dialkylsydnones were significantly lower than for the 3-alkylsydnones. In addition, the CD_{50} values for the 3,4-dialkylsydnones were close to one another and were independent of the partition coefficient.

PRELIMINARY WORK in this laboratory (1) indicated that 3-*sec*-butylsyndone (I, R = *sec*-Bu, R′ = H)



(I)

was a potent central nervous system stimulant with a particularly stimulating effect on respiration. It did not potentiate acetylcholine, nor did it produce acetylcholinesterase inhibition in these studies. It produced a more favorable respiratory response and blood pressure rise than the same dose of pentylenetetrazole when administered intravenously to a pentobarbitalized dog.

With these preliminary findings in hand, a systematic study of a number of sydnones was initiated with the purpose of further elaborating the pharmacological properties and any structure-activity relationships. In the previous communication from this laboratory (2), a number of 3-alkylsydnones were synthesized. The pharmacological response of these compounds was qualitatively similar to that of 3-*sec*-butylsydnone, but one response in particular—the onset of a charac-

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teristic convulsive pattern—varied appreciably concerning the minimum dose necessary to initiate it. One physical parameter of these compounds was closely correlated with the convulsive dose, that of the oil-water partition coefficient. If the pharmacological action was chiefly central, as preliminary data indicated, then the sydnones with a greater affinity for lipid solvents would be expected to penetrate the lipid barrier into the CNS with greater facility. This was precisely the case for the 3-alkylsydnones. Those with a higher partition coefficient required a lower dose (mmoles/Kg.) to bring on convulsions in 50% of the animals (CD_{50}).

This current study was initiated with several objectives in mind. First, since the previous study dealt only with the 3-alkylsydnones, the effect on activity by substitution at both 3 and 4 positions should be assessed. Second, the study would afford a comparison between the two series with respect to pharmacological activity and physical properties. Finally, a more detailed pharmacological evaluation of representative members of both groups would be conducted and some generalizations made regarding the activity of the ring itself and structural features enhancing this activity.

A group of 3,4-dialkylsydnones was selected in order to maintain a relatively short chain length at both the 3 and 4 position to achieve partition coefficients approximating those of the 3-alkyl series.

Essentially the same method of synthesis was employed in this series as that previously reported (3). Trifluoroacetic anhydride was tried as a ring closing reagent with III in the final step of the synthesis, but this did not improve the yield and, in fact, frequently resulted in a lower yield of sydnone. (See Tables I and II). Generally, ring closure to form dialkylsydnones proceeded with greater difficulty than was encountered with the 3-alkylsydnones. This is quite probably due to the inductive effect of the 4-alkyl group which lowers the acidity of the 4-hydrogen atom in IV. This in turn would result in a higher activation necessary for its removal from the transition state (II) based upon Baker's proposed mechanism (4).

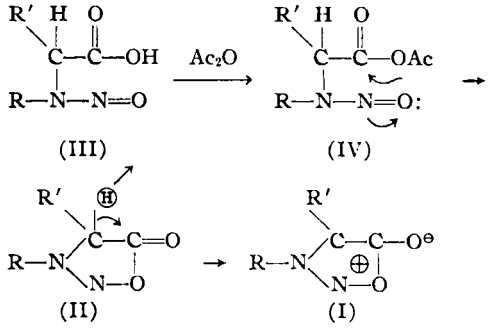
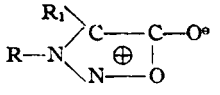


TABLE I.—ALKYLAMINOESTERS

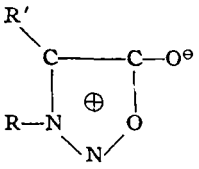
Name	Formula	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	B.p.	Yield, %
Methyl *N*-cyclohexyl-aminopropionate	$C_{10}H_{19}O_2N$	64.83	10.34	7.56	64.65	10.16	7.56	75°/1mm.	51
Methyl *N*-ethylamino-propionate	$C_6H_{13}O_2N$	54.94	9.99	10.68	55.23	9.79	10.53	61°/5mm.	51
Ethyl *N*-*tert*-butylamino-propionate	$C_9H_{19}O_2N$	62.39	11.05	8.09	61.91	11.24	8.16	100°/20mm.	41
Ethyl *N*-butylamino-propionate	$C_9H_{19}O_2N$	62.39	11.05	8.09	62.41	10.82	7.91	59°/10mm.	47
Ethyl *N*-propylamino-butyrate	$C_9H_{19}O_2N$	62.39	11.05	8.09	62.31	10.86	8.28	60°/5mm.	53
Methyl *N*-butylamino-butyrate	$C_9H_{19}O_2N$	62.39	11.05	8.09	62.41	10.82	7.91	81°/10mm.	47
Ethyl *N*-butylamino-hexanoate	$C_{12}H_{25}O_2N$	66.93	11.70	6.51	67.08	11.52	6.91	84°/3mm.	27

TABLE II.—3,4-DIALKYLSYDNONE



R	R_1	Formula	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	λC–O I.R.	U.V. max.	U.V. E.	B.p. or m.p.	Yield %
Cyclohexyl	CH_3	$C_9H_{14}O_2N_2$	59.32	7.69	15.38	59.41	7.82	15.16	5.83	294	6652	151°	53
Ethyl	CH_3	$C_6H_8O_2N_2$	46.87	6.29	21.87	47.07	6.82	21.96	5.80	295	5866	133°/0.5mm.	40
tert-Butyl	CH_3	$C_7H_{12}O_2N_2$	53.83	7.74	17.94	53.67	7.91	17.91	5.78	293	5850	153°	50
Butyl	CH_3	$C_7H_{12}O_2N_2$	53.83	7.74	17.94	53.93	7.89	18.03	5.80	295	6441	149°	31
Propyl	C_2H_5	$C_7H_{12}O_2N_2$	53.83	7.74	17.94	53.92	7.71	17.62	5.79	293	6028	128°/5mm.	35
Butyl	C_2H_5	$C_8H_{14}O_2N_2$	56.45	8.29	15.46	56.74	8.40	15.51	5.77	296	6658	130°/4mm.	41
Propyl	CH_3	$C_5H_{10}O_2N_2$	50.69	7.04	19.71	51.19	7.68	19.88	5.80	295	5905	135°/13mm.	48

TABLE III.—SYDNONE PHYSICAL AND PHARMACOLOGICAL DATA



R	R′	PC (*n*-Amyl Alcohol) H_2O	CD_{50} mmole/Kg.	R	R′	PC (*n*-Amyl Alcohol) H_2O	CD_{50} mmole/Kg.
Isopropyl	H	2.3	2.96	Cyclohexyl	CH_3	1.85	0.24
Propyl	H	2.5	1.60	Ethyl	CH_3	2.05	...
tert-Butyl	H	4.9	0.87	Propyl	CH_3	2.8	0.34
sec-Butyl	H	6.4	1.13	Butyl	CH_3	2.9	0.13
tert-Octyl	H	8.0	0.25	Butyl	C_2H_5	4.1	0.19
				tert-Butyl	CH_3	6.7	0.71

The partition coefficients of the 3,4-dialkylsydnones could not be satisfactorily run in chloroform in order to compare these values with those obtained for the 3-alkylsydnones; hence, both series were run in amyl alcohol. The CD_{50} values were obtained for the 3,4-dialkylsydnones as previously described (5). (See Table III).

A comparison of the CD_{50} values for both sydnone series and their partition coefficients revealed some significant differences. Although both series of compounds were similar in their central convulsive activity, the CD_{50} values for the 3,4-dialkylsydnones were significantly lower than the 3-alkylsydnone values. (See Fig. 1). In addition, the CD_{50} values for the 3,4-dialkylsydnones appeared to be very close to one another and were independent of the partition coefficient.

One possible explanation for this difference is that in the case of the 3-alkylsydnones, the absolute lipid solubility is sufficiently low so that the partition coefficient becomes a limiting factor concerning the amount of drug which reaches the CNS. In the case of the 3,4-dialkylsydnones, the absolute lipid solubility is appreciably higher so that in spite of a less favorable partition coefficient in some compounds, there is sufficient solubility in the biolipid phase to permit an active level of sydnone to reach the site of action to elicit a response. In other words, because of a high-absolute solubility in the biolipid phase, the partition coefficient of the 3,4-dialkylsydnones is not a limiting factor to activity.

On the other hand, if the 3,4-dialkylsydnones are not more soluble in the lipid phase, then an alternative explanation is that the intrinsic activity of the 3,4-dialkylsydnones is greater than that for the 3-alkylsydnones. Thus throughout the range of partition coefficient values studied for the 3,4-dialkylsydnones, there is sufficient drug being absorbed to elicit a response. This minimal amount necessary to elicit a response must then be less than the amount which is possible to be absorbed with a given partition coefficient value.

A more detailed pharmacological study of these compounds will be published elsewhere (6). This study revealed that representative members of both groups possessed diuretic activity as well as a chloruretic, naturetic, and glucosuretic effect. In a comparative study, 3-*sec*-butylsydnone had a moderate effect in lowering the blood pressure of a dog and was

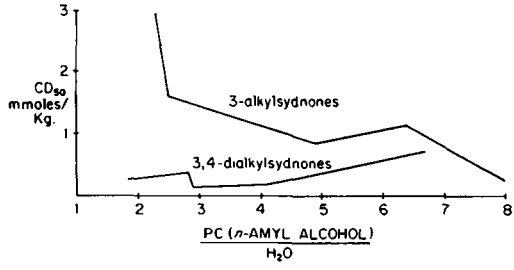


Fig. 1.—Partition coefficients *vs.* minimum convulsive dose of 3-alkyl- and 3,4-dialkylsydnones.

superior to 3-butyl-4-ethylsydnone in this regard. In higher doses, both compounds caused respiratory difficulty, labored breathing, and lung hemorrhages.

EXPERIMENTAL[1]

Alkylaminoester Synthesis.—Since all of these syntheses were essentially the same, the synthesis of methyl *N*-ethylaminopropionate is described as typical. A solution of methyl α-bromopropionate (83.5 Gm., 0.5 mole) was added dropwise to a solution of ethylamine (45.09 Gm., 1.0 mole) dissolved in dry benzene. The mixture was stirred for 1 hour, then refluxed for 2 hours. The ethylamine hydrobromide was filtered out and the benzene removed from the filtrate under vacuum. The residue was distilled, b.p. 60°/5 mm., yield 51%. See Table I for physical constants, yields, and analyses.

Sydnone Synthesis.—The synthesis of 3-ethyl-4-methylsydnone is described as a typical example. Twenty grams of methyl *N*-ethylaminopropionate was hydrolyzed by refluxing with 14 Gm. of sodium hydroxide in 200 ml. of water. The solution was then cooled to −5° and made acid to pH 2. Sixteen grams of sodium nitrite dissolved in 50 ml. of water was added to this reaction mixture. The addition was made slowly to keep the temperature below 0°. This was stirred for 3 hours, then extracted with ether. The residue from the ether gave a positive Liebermann nitroso test. The nitrosoaminoacid was not isolated but was treated directly with 150 ml.

[1] All melting points were done on the Kofler apparatus and are corrected. Analyses were performed by Weiler and Strauss Laboratories, Oxford, England, and by Galbraith Laboratories, Knoxville, Tenn.

of freshly distilled acetic anhydride and allowed to stand in the dark for 2 days. The volatile products were then removed under vacuum, and the last traces of acetic acid were removed by azeotropic distillation with xylene. The remaining oil was distilled under vacuum, b.p. 133–135°/0.5 mm., yield 40%. (See Table II for physical constants, yields, and analyses.)

Partition Coefficients.—The partition coefficients of both the 3-alkylsydnones and the 3,4-dialkylsydnones were obtained between water saturated *n*-amyl alcohol and *n*-amyl alcohol saturated water. Several concentrations of each compound in water saturated *n*-amyl alcohol were equilibrated at 23° with varying quantities of *n*-amyl alcohol saturated water. The concentration in the *n*-amyl alcohol before and after equilibration with the aqueous phase was determined from the ultraviolet absorption at the maximum. An average of at least four determinations for each compound was used. (See Table III).

Determination of CD_{50} Values.—The same procedure previously referred to (5) was used to determine the CD_{50} values. The CD_{50} values for the 3-alkylsydnones were reported in a previous communication (2). (See Table III). The partition coefficient *versus* CD_{50} values are graphically portrayed in Fig. 1.

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Color Reactions of Veratrum Alkaloids with Sulfuric Acid and Sulfuric Acid Reagents

By HORACE D. GRAHAM†

Veratrum alkaloids, when treated with concentrated sulfuric acid, or "sulfuric acid reagents" give red, purple, yellow, or violet with characteristic absorption maxima. This reaction can be used as the basis of a simple, rapid colorimetric method for the determination of isolated samples of this class of alkaloids. The final concentration of sulfuric acid is very critical for maximum color development and, since color intensity is decreased if water is present to the extent of greater than 1 per cent, an anhydrous medium is recommended for maximum development. The typical color is best obtained when ethyl alcohol or methyl alcohol is used as the solvent for the alkaloids. Except for hydrochloric acid, no color is obtained with acids other than sulfuric. After heating for several minutes in hydrochloric acid, some color developed, but the intensity was always much less than that produced after treatment of the same amount of alkaloid with sulfuric acid. Inorganic salts, sodium benzoate, and glucose, above certain limiting concentrations, will interfere with color development.

CONSIDERABLE INTEREST has developed in the veratrum alkaloids because of their potent hypotensive action. This was forcibly emphasized by Kupchan (1), who recently reviewed the hypotensive veratrum ester alkaloids, paying particular attention to the relationship between structure and hypotensive activity. However, despite their growing importance in medicine and pharmacology, simple, rapid methods for the quantitative assay of veratrum alkaloids are still lacking. As far as can be ascertained, present methods of detection and assay involve mainly biological means such as the pigeon emetic response or intricate physical, chemical, or other methods (2–18). Dadlez, *et al.* (19), reported on the color reactions of alkaloids, including veratrine, with sulfuric acid and sulfuric acid in combination with several other reagents such as furfural, *p*-dimethylaminobenzaldehyde, and vanillin. Moraes and Palma (20) employed both concentrated nitric acid and concentrated sulfuric acid in studying the color reactions of several alkaloids including veratrine, following separation of the alkaloids by paper chromatography. Mandelin reagent (concentrated sulfuric acid plus formalin) and Fröehde reagent (concentrated sulfuric acid plus ammonium molybdate) were also used.

Reference has been made (21) to the red, purple, or violet developed by certain veratrum alkaloids on treatment with concentrated sulfuric acid. Since alkaloids of many other classes do not give these colors, a study of the reaction was undertaken to delineate those factors which

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