formed long yellow birefringent prisms, m.p. 162–164° (sintering at 153°, yellow-green melt).

Anal. Calcd. for $C_{13}H_{16}N\cdot C_6H_3N_3O_7$: C, 54.94; H, 4.60. Found: C, 54.99; H, 4.94.

Rearrangement to 9-Methylcarbazole (XXII).—When the free alkamine (XIX) is left in a mixture of methanol and concentrated hydrochloric acid at room temperature, the unchanged free base can be recovered. With methanolic concentrated HCl (1:1) on the steam-bath or in polyphosphoric acid at 150° for a few minutes the alkamine is rearranged to a non-basic compound which crystallizes out of the acid- and base-washed and dried ether extract in colorless plates, m.p. 48°. The ultraviolet and infrared spectra were in agreement with an N-methyl indole compound. The picrate, prepared in benzene solution, formed red-brown needles, m.p. 114°.

The literature records m.p. 50°[46,47] for 9-methyltetrahydrocarbazole and 114.5°[46] and 116°[47] for the picrate.

N-Methylgelsemine.—A mixture of 2.41 g. of gelsemine, 300 mg. of potassium metal and 30 cc. of benzene was refluxed and stirred. Within 15 minutes a copious creamy precipitate had formed. After an hour 1.09 g. of methyl iodide in 4 cc. of benzene was added and refluxing was continued for two more hours. After standing overnight the mixture was filtered. Evaporation of the filtrate (steam-bath) left 552 mg. (22%) of a yellow oil which crystallized on scratching. Decomposition of the filter cake with water, followed by ether extraction, recovered 275 mg. of gelsemine, bringing the corrected yield to 25%. The crude N-methyl compound was taken up in boiling ligroin, decolorized with diatomaceous earth, and allowed to crystallize; colorless prisms, m.p. 141–142.5° (sublimes in small prisms 126°; clear colorless melt).

Anal. Calcd. for $C_{21}H_{24}N_2O_2$: C, 75.01; H, 7.20; N, 8.33. Found: C, 74.72; H, 7.27; N, 8.23.

The reduction of N-methylgelsemine with lithium aluminum hydride gave N-methyldesoxydihydrogelsemine, m.p. 117–119°, identical with des-N-methyldesoxydihydrogelsemine.[35]

N-Methyldihydrogelsemine. A. By Hydrogenation.—A solution of 50 mg. of N-methylgelsemine in 4 cc. of methanol was stirred with 20 mg. of platinum oxide under hydrogen. In 19 minutes the hydrogen uptake was 21.65 cc. The solution was filtered and evaporated to leave colorless birefringent prisms, m.p. 164–168° identical (infrared spectrum) with the product obtained by methylation of dihydrogelsemine.

B. By **Methylation.**—A mixture of 213 mg. of dihydrogelsemine, 40 mg. of metallic potassium and 20 cc. of benzene was refluxed for 4 hours, 120 mg. of methyl iodide in 2 cc. of benzene was added dropwise and refluxing continued overnight. The mixture was filtered and the filtrate evaporated to leave 260 mg. (80%) of a yellow oil which crystallized readily on trituration with hexane. On recrystallization from hexane (and decolorization with diatomaceous earth) there were obtained large colorless birefringent prisms, m.p. 167–169° (sublimes in long needles 134°, sinters 157°, clear colorless melt).

Anal. Calcd. for $C_{21}H_{26}N_2O_2$: C, 74.53; H, 7.75; N, 8.28. Found: C, 75.17; H, 8.18; N, 8.12.

The reduction of N-methyldihydrogelsemine with lithium aluminum hydride led to N-methyltetrahydrodesoxygelsemine, m.p. 148°, identical with des-N-methyl-desoxytetrahydrogelsemine.[35]

Methiodide.—A mixture of 200 mg. of N-methyldihydrogelsemine, 10 cc. of methanol and 0.5 cc. of methyl iodide was refluxed for 30 minutes, left overnight, and evaporated on the steam-bath. The residue, amounting to 300 mg., crystallized readily on trituration with acetone. After washing with acetone, a portion was recrystallized from ethanol. Small hard glossy birefringent cubes, m.p. 305–306.5° (slow darkening from 200°, spalling and crystalline transformation 250–265°, melts with decomposition, brown melt).

Anal. Calcd. for $C_{22}H_{29}N_2O_2I$: C, 55.00; H, 6.08; N, 5.83. Found: C, 55.34; H, 6.07; N, 5.78.

BETHESDA, MARYLAND

(46) H. Adkins and H. L. Coonradt, THIS JOURNAL, **63**, 1563 (1941).

(47) W. H. Perkin and S. G. P. Plant, *J. Chem. Soc.*, **119**, 1834 (1921).

[CONTRIBUTION FROM THE RESEARCH AND DEVELOPMENT DIVISION OF SMITH, KLINE AND FRENCH LABORATORIES]

C^{14}-Labeled Colchicine Derivatives[1]

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RECEIVED DECEMBER 22, 1952

Six derivatives of colchicine, each labeled with C^{14} in a known position of the molecule, have been prepared. As an adjunct to the preparations involving labeled diazomethane, a method was developed for the quantitative estimation of this reagent in solutions containing it based upon the well-known ferric reaction of colchiceine.

Discussion

In order to study the fate of colchicine derivatives in biological studies, the preparation of a series of representative compounds labeled with C^{14} was undertaken. Walaszek[3] recently reported the biosynthesis of such compounds by growth of colchicum plants in an atmosphere of $C^{14}O_2$. Extraction of the plants gave the C^{14}-alkaloids. With this method, however, the positions of the labeled carbon atoms are not known, and thus the identification of labeled metabolic products is more difficult. Through the use of radioactive diazomethane and radioactive acetyl chloride we have prepared a number of colchicine derivatives labeled in rings A, B and C as shown in formulas I, II and III.

Ordinarily, in reactions involving the use of diazomethane, a liberal excess of the reagent is employed. In the present problem, however, it became necessary to obtain maximum methylation with a minimum expenditure of the C^{14} reagent. This involved the determination of the diazomethane equivalent of the nitrosomethylurea, optimum conditions for the methylation of the various colchicine derivatives, and adequate separation of isomeric products when these were formed.

The usual methods[4] for the determination of diazomethane were unsuitable inasmuch as they would have consumed the reagent to give C^{14} products of no immediate interest to our problem. A method was therefore devised based on the well-

(1) This investigation was supported (in part) by a research grant from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

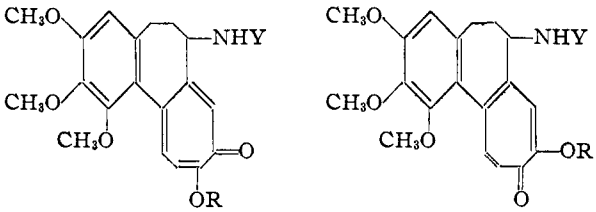
(2) Research Associate.

(3) E. J. Walaszek, F. E. Kelsey and E. M. K. Geiling, *Science*, **116**, 225 (1952).

(4) A. H. Blatt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 165.

known ferric reaction of colchiceine[5] in which this compound gives a deep green color with ferric chloride whereas its methylated derivative, colchicine, does not. Experience showed: (1) that the intensity of the color was proportional to the concentration of colchiceine in solution within the limits of practical use (10^{-3} to 10^{-4} millimole); (2) that after an initial 10-minute period of development the color was stable enough to allow colorimetric comparison; (3) that the presence of colchicine in amounts (1 to 3 $\times$ 10^{-3} millimole/10 cc.) anticipated in actual experiment did not interfere with the development nor with the intensity of the color. Thus, by allowing a solution of diazomethane to react with an excess of colchiceine and measuring the amount of unmethylated material remaining by colorimetric comparison with colchiceine solutions of known concentration, the amount of diazomethane could be readily calculated. The method gave results in good agreement with those obtained by the benzoic acid method using four different samples of nitrosomethylurea.

The acidic nature of the free hydroxyl group on the tropolone ring of colchiceine [(I $\leftrightarrows$ II), R = H, Y = $COCH_3$] and trimethylcolchicinic acid [(I $\leftrightarrows$ II), R = H, Y = H] makes for rather easy methylation of these compounds. Solutions of them in methylene chloride were added to a slight excess of diazomethane in ether, and methylation was allowed to proceed at 0–5°. After removal of the solvents and excess diazomethane by distillation, the products no longer gave a positive test with ferric chloride. Desmethylcolchicine[5a] (III, R = H), which contains a phenolic hydroxyl in ring A, is more difficult to methylate. In this case the reaction was forced by gently refluxing the solution in ether–diazomethane using a cold-finger condenser cooled with Dry Ice–acetone. A liberal excess of the reagent was necessary for complete methylation.



I
Normal series

II
Iso series

$$[\text{I} \leftrightarrows \text{II}] + {}^{*}CH_2N_2 \longrightarrow \text{I} + \text{II}$$

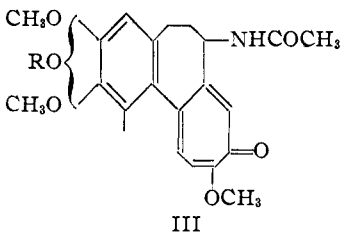
R = H, Y = $COCH_3$ R = $^{*}CH_3$–, Y = $COCH_3$

$$[\text{I} \leftrightarrows \text{II}] + {}^{*}CH_2N_2 \longrightarrow \text{I} + \text{II}$$

R = H, Y = H R = $^{*}CH_3$–, Y = H

$$\text{I} + CH_3{}^{*}COCl \longrightarrow \text{I}$$

R = CH_3, Y = H R = CH_3–, Y = $^{*}COCH_3$



III

$$\text{III} + {}^{*}CH_2N_2 \longrightarrow \text{III}$$

R = H R = $^{*}CH_3$

The separation of isomeric products obtained as a result of the methylation of colchiceine[6] and trimethylcolchicinic acid[7] was accomplished on neutral alumina columns by the fluid chromatogram technique. Chloroform and chloroform–methanol were the eluants of choice. The chloroform (reagent grade) was freed from ethanol (preservative); the alumina was washed free of alkali with hot water, then washed with alcohol and dried by heating *in vacuo* for one-half hour at 185°. The forced methylation of III (R = H) led to a small amount of highly colored, amorphous by-product which was readily removed by passage of the methylated mixture through a column of alumina.

The specific activity of the final products was determined by C^{14} count on "infinitely thin" layers by the flow counting technique with an estimated error of 5%.[8]

Acknowledgment.—We are indebted to Mr. Morris A. Leaffer and Mr. James A. Gibbs of Tracerlab, Inc., for their valuable assistance in carrying out portions of the experimental work involving radioactive reagents.

Experimental

A. **Colchicine and Isocolchicine from Colchiceine.** (1) **Estimation of Diazomethane Content of Nitrosomethylurea.**—A 100-cc. distilling flask (a) was fitted with a long side-arm bent so that its lower end could be inserted into the bulb of a second 100-cc. distilling flask used as a receiver (b). The receiver had a shortened side-arm to which a bubble-counter trap (c) or a bent side-arm (d) could be attached with a short piece of rubber tubing. The flask (a) was cooled by means of an ice-bath and the receiver (b) was immersed in a Dry Ice–acetone cooling mixture. The receiver was fitted with a trap (c) containing 0.5 cc. of glacial acetic acid. To (a) were then added 5 cc. of 40% KOH and 25–30 cc. of ether in which 103 mg. (1 millimole) of nitrosomethylurea had been dissolved. The flask was shaken from time to time until the generation of diazomethane appeared complete. The ice-bath was replaced by a warm (50°) water-bath and the diazomethane solution was distilled into the receiver. The receiver was disconnected, leaving the acetic acid trap in place, and placed in an ice-bath. By means of a separatory funnel fitted with a rubber stopper to the neck of the flask (b), a solution of 385 mg. (1 millimole) of colchiceine in methylene chloride was added and the stoppered flask was kept at 0–5° for 1 hour. The solution was then transferred to a glass-stoppered graduated cylinder and diluted to 50 cc. with methylene chloride.

By means of a pipet, 0.1 cc. of this solution was transferred to a 10-cc. Klett colorimeter cell, the solvent was removed by agitation in the water-bath and the residue was dissolved in a few drops of methanol and diluted almost to the mark with water. In a second colorimeter cell was placed 0.5 cc. of a standard colchiceine solution (77 mg./100 cc.) in methylene chloride and, by the same procedure, this too was diluted. In a third tube was placed 9.5 cc. of dis-

(5) (a) M. L. Oberlin, *Ann. chim. phys.*, [3] **50**, 108 (1857); (b) E. Boyland and M. E. Boyland, *Biochem. J.*, **31**, 454 (1937); (c) E. Boyland and E. H. Mawson, *ibid.*, **32**, 1204 (1938).

(5a) Note added in proof: Šantavý, *Chem. Listy*, **46**, 280 (1952), has shown that the hydroxyl group occupies position 2.

(6) M. Sorkin, *Helv. Chim. Acta*, **29**, 246 (1946).

(7) The methylation and other transformations of trimethylcolchicinic acid will be described in greater detail in a subsequent publication.

(8) C^{14} Analyses by Tracerlab Inc., Boston, Mass.

tilled water. To each of the three tubes was then added 2 drops of 0.1 *N* sulfuric acid and 4 drops of 1% ferric chloride solution, and the volume was adjusted to 10 cc.

The contents of each tube were mixed and the colors developed were compared, after 10 minutes, in the Klett colorimeter using a No. 42 filter. The amount of unreacted colchiceine, and therefore the amount of diazomethane corresponding to 1 millimole of nitrosomethylurea, was calculated.

Final colchiceine standard	10^{-3} millimole
Colorimeter reading, standard	218
Colorimeter reading, sample	190, 188
Total calcd. colchiceine in sample	0.435 millimole
Yield diazomethane	56.5%

(2) **Methylation of Colchiceine.**—The remainder of the partially methylated colchiceine from (1) was returned to the receiving flask (b) and another millimole (385 mg.) of colchiceine was added. (The total amount was thus 1.43 millimoles). The solution was concentrated *in vacuo* to about 20 cc. Sufficient nitrosomethylurea was taken to generate, in the manner already described, 2 millimoles of diazomethane and the reaction was allowed to proceed at 0–5° overnight. The flask was then converted to a distilling flask by attachment of the side-arm (d), a second Dry Ice–acetone cooled receiver was attached, and the excess diazomethane was distilled from the solution as previously described. The methylated mixture was then removed; it did not give a ferric chloride test as described under (1) above.

The solvents were removed by evaporation *in vacuo*, the residue was taken up in chloroform and transferred to a column of 24 g. of neutral alumina. The chromatogram was developed using 80-cc. portions of solvents. Elution by chloroform–0.5% methanol gave two fractions which, after removal of solvent and crystallization from ethyl acetate–ether, yielded (a) 310 mg. of colchicine, m.p. 155–160°,[9] and (b) 250 mg. of a mixture of colchicine and isocolchicine, m.p. 155–170°. Elution with chloroform–1.0% methanol gave 30 mg. of isocolchicine, m.p. 216–218°. The combined residues obtained by evaporation of the mother liquors from these crystallizations weighed 130 mg.

B. **Methylation of Desmethylcolchicine.**—In the manner previously described, 13.6 millimoles of nitrosomethylurea was taken to prepare a solution of diazomethane in ether. The distillate was collected in the flask containing the excess from (2) above. The receiver was removed, the acetic acid trap was detached and the side-arm of the flask was stoppered with a small rubber stopper. After addition of a solution of 2 millimoles (770 mg.) of desmethylcolchicine in methylene chloride containing a little methanol (*ca.* 10%) the flask was fitted with a cold-finger reflux condenser containing a slush of Dry Ice and acetone. A trap containing ether and cooled to −80° was attached to the condenser outlet, and by means of a warm water-bath the solution was caused to reflux gently for 1 hour. After cooling somewhat, the flask was reconverted to a distilling flask and the excess diazomethane was removed in the usual manner. The product, after removal of solvent *in vacuo*, was taken up in chloroform–0.5% methanol and purified by passage through a small (1 × 10 cm.) column of slightly alkaline alumina. Removal of solvent gave a residue which crystallized from ethyl acetate–ether to yield 510 mg. of colchicine, m.p. 154–158°; the mother liquors gave 180 mg. of residue after evaporation. An additional quantity of an orange amorphous material was eluted from the column by methanol. It was not further investigated.

(9) R. M. Horowitz and G. E. Ullyot, THIS JOURNAL, **74**, 587 (1952).

C. **Methylation of Trimethylcolchicinic Acid.**—The diazomethane recovered from the previous reaction was allowed to react with an excess (1.07 g., 3 millimoles) of trimethylcolchicinic acid in methylene chloride (this excess was estimated since the amount of diazomethane present at this point was not accurately known). After standing for four hours at 0–5° the solvent was evaporated *in vacuo*. The residue consisting of crude trimethylcolchicinic acid methyl ethers and unmethylated starting material was taken up in dilute (0.1 *N*) sodium hydroxide and extracted twice with chloroform. The chloroform layer was washed with water and evaporated to dryness *in vacuo*. The residue was dissolved in ethanol, a slight excess of *d*-tartaric acid was added, the mixture was heated on the hot-plate for a few minutes, and was then allowed to stand at room temperature overnight. The product was removed by filtration, washed with alcohol and dried. The light yellow microcrystalline powder weighed 630 mg. and melted at 206–208° with decomposition. It was recrystallized from alcohol–water; m.p. 213–216° dec.

The crude isotrimethylcolchicinic acid methyl ether *d*-tartrate (250 mg.) was obtained by concentration of the filtrates and mother liquors of the *n*-compound and crystallization from methanol–acetone; m.p. 160–170° dec.

D. **Acetylation of *n*-Trimethylcolchicinic Acid Methyl Ether.**—A sample (1.0 g., 2.8 millimoles) of *n*-trimethylcolchicinic acid methyl ether, as a solution in pyridine, was transferred to a Claisen flask modified as follows: the center neck, equipped with standard-taper joint, contained a ring seal which could be broken by a small metal hammer operated from outside the flask by a hand magnet; the side-arm, likewise equipped with a standard-taper joint, was constricted slightly for easy sealing. The flask was attached to a high vacuum manifold by means of the side-arm and the contents of the flask were frozen by means of liquid nitrogen. From a similar flask attached to the manifold 3.5 millimoles of acetyl chloride was distilled into the mixture. The side-arm of the reaction flask was sealed at the constriction and the flask was allowed to come to room temperature. It was then warmed to 55–60° for 15 min., cooled, and the contents were refrozen. The flask was returned to the manifold, the seal was broken, and the solvents were removed by distillation. The residue was dissolved in chloroform and the solution was washed with water. The chloroform was removed and the residue was dried *in vacuo*. The crude product, which weighed 1060 mg., was dissolved in chloroform–0.5% methanol and passed through a 1 × 10 cm. column of neutral alumina. After removal of solvent and crystallization of the residue from ethyl acetate–ether, 780 mg. of colchicine, m.p. 153–155°, was obtained. The residues remaining after evaporation of the mother liquors weighed 130 mg.

Identical experiments using C^{14}-nitrosomethylurea (specific activity *ca.* 1 mc./millimole) which yielded 57% diazomethane by the analytical procedure described gave, in essentially the same yields, the following products: colchicine tagged in ring A (III, R = $\overset{*}{C}H_3$–); ring C labeled colchicine (I, R = CH_3, Y = $COCH_3$) and isocolchicine (II, R = $\overset{*}{C}H_3$–, Y = $COCH_3$–); ring C labeled trimethyl colchicinic acid methyl ether (I, R = $\overset{*}{C}H_3$–, Y = H) and its *iso*-derivative (II, R = $\overset{*}{C}H_3$–, Y = H). These products had a specific activity of 1.03 mc./millimole. Two acetylations using C^{14}-acetyl chloride gave ring B labeled colchicine (I, R = CH_3, Y = $-\overset{*}{C}OCH_3$) with specific activities of 0.92 and 1.02 mc./millimole.

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