was used. Usually, chromatographic separation plays a central role time is devoted to sample isolation and purification.

3a,4,5,6-Tetrahydro-3a-acetyl-5,5-dimethyl-1H-inden-1-one (5). A solution of 103 mg (0.46 mmol) of 1 and 2 mg of p-toluene-sulfonic acid in 17 mL of dry benzene was heated at reflux for 4.5 h with azeotropic removal of the water produced. The reaction mixture was then cooled to room temperature, and 3 mL of saturated aqueous sodium bicarbonate was added. The mixture was extracted with EtOAc, and the combined ether layers were dried (MgSO4) and concentrated. The crude product was purified by chromatography on silica gel. Elution with 2:1 hexanes–ether gave 14 mg (15%) of 4 and 43 mg (45%) of the crystalline product 5: mp 65–67°C; Rf 0.81 (ether); IR (CCl4) 1710, 1657 cm–1; 1H NMR (400 MHz, CDCl3) δ 7.13 (d, 1H, J = 5.90 Hz), 6.78 (t, 1H, J = 4.40 Hz), 6.43 (d, 1H, J = 5.90 Hz), 2.65 (d, 1H, Jαm = 12.94 Hz), 2.08 (d, 1H, J = 4.40 Hz), 2.02 (s, 3H), 1.16 (d, 1H, J = 12.94 Hz), 0.99 (s, 3H), 0.90 (s, 3H); 13C NMR (CDCl3) δ 205.87, 195.03, 185.67, 140.10, 136.79, 134.58, 130.67, 128.19, 127.32, 126.10, 124.10, 119.48, 118.51, 113.15, 112.95, 111.32, 109.49, 79.36, 37.86, 26.78, 25.86, 22.91, 22.65, 18.94, 13.75 Hz; 2.2 Hz), 3.64 (td, 1H, J = 11.70, 2.20 Hz), 2.26 (dd, 1H, J = 18.67 Hz), 2.60 (dd, 1H, J = 19.25, 9.35, 1.0 Hz), 2.40 (d, 1H, Jαm = 13.75 Hz), 2.33 (dd, 1H, J = 11.20, 2.20 Hz), 2.26 (dd, 1H, J = 18.67 Hz), 2.25 (s, 3H), 2.14 (d, 1H, Jαm = 19.25 Hz), 1.78 (d, 1H, Jαm = 13.75 Hz), 1.31 (s, 3H), 0.35 (s, 3H); 13C NMR (CDCl3) δ 215.07, 208.45, 201.98, 67.77, 60.10, 53.02, 48.93, 43.31, 42.43, 40.64, 39.22, 25.53, 23.70.

The steps to follow in packing a column are detailed in Figure 1 (Figures 1–3, with accompanying legends describing details of column preparation and operation, are available as supplementary material). Note that the silica gel bed is first allowed to settle by gravity flow and then further compacted by application of air pressure. This assures a dense, evenly packed bed. Then, rather than application of the mixture to be chromatographed in liquid form, it is first evaporated onto coarse silica gel. This assures even application of the sample onto the top of the column and avoids concerns about mixtures that are not soluble in the (usually nonpolar) column solvent.

We find it convenient in running such columns to adjust the air pressure so as to collect about one fraction per minute. Fractions are monitored by TLC. For routine separations, the polarity of the eluant is adjusted so that the first component of the mixture appears in about fraction 10. It is usually then sufficient to collect 20 fractions, with fraction collection and TLC monitoring being effected simultaneously. When components of the mixture are widely separated, it is appropriate to switch to a more polar eluant after the less polar components have come off the column. The entire process of column construction, elution, and fraction analysis usually takes a little less than 1 h.

We have used a variety of solvent mixtures following this procedure. Ethyl acetate in petroleum ether appears to be the most generally satisfactory. For less polar mixtures, CH2Cl2 in petroleum ether is effective, and for very polar mixtures we use ethyl acetate in CH2Cl2. We have found that if it requires more than 40% ethyl acetate in hexane or less than 5% to give a TLC Rf of 0.4 for the mixture to be separated, it is best to switch to the alternative less polar or more polar solvent system. While it is possible to plot "most effective column eluant" as a function of TLC Rf, derivation of the most effective solvent system for a given separation is still best done empirically.

Acknowledgment. Grateful acknowledgment is extended to the donors of the Petroleum Research Fund, administered by the American Chemical Society, to Research Corp., to the National Institutes of Health, and to the American Cancer Society (Grant IN-107F, awarded to C.W.M.) for their generous support for this research. High-field NMR spectra were obtained through the National Science Foundation Regional NMR Center at the University of South Carolina (Grant CHE 78-18723).

Registry No. 1, 80594-76-9; 4, 80594-77-0; 5, 80594-78-1; 6, 80695-33-6.

TLC Mesh Column Chromatography

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Received July 6, 1981

In a usual laboratory day, the greatest share of working time is devoted to sample isolation and purification. Usually, chromatographic separation plays a central role in this effort. We outline here a procedure for column chromatography that is both efficient (mixtures showing ∆Rf = 0.05 by TLC are routinely separated) and easily scaled up.

There are two central concerns in column chromatography: packing of the absorptive bed and sample application. This procedure, a modification of the short-column technique, effectively addresses both of these concerns.

We have used a variety of solvent mixtures following this procedure. Ethyl acetate in petroleum ether appears to be the most generally satisfactory. For less polar mixtures, CH2Cl2 in petroleum ether is effective, and for very polar mixtures we use ethyl acetate in CH2Cl2. We have found that if it requires more than 40% ethyl acetate in hexane or less than 5% to give a TLC Rf of 0.4 for the mixture to be separated, it is best to switch to the alternative less polar or more polar solvent system. While it is possible to plot "most effective column eluant" as a function of TLC Rf, derivation of the most effective solvent system for a given separation is still best done empirically.

(1) EM 7747 silica gel (10–15 μm), purchased from Scientific Products, was used.
Typical column sizes used are shown in Table I. The five smaller columns are packed and run as described in Figure 1. For the four smallest, commercial columns are used as received. Air pressure is introduced through a glass tube inserted through a one-hole rubber stopper in the top of the column (Figure 2). It is convenient to maintain column pressure with laboratory compressed air, delivered through a length of Tygon tubing having a small syringe needle inserted in it for a bleed. The same procedure is followed for the 50-g column, except that the top of the commercial column is modified by attaching to it a female 35/20 ball joint. The male joint is necked down to a tubing connector for the air line and secured to the female joint with a screw clamp (Figure 2). The three largest columns are also packed and run as described, except that aspirator vacuum is substituted for air pressure. Fractions are collected in Erlenmeyer flasks by using a vacuum adapter as shown in Figure 3. Again, it is important to close the stopcock at the bottom of the column before releasing the vacuum to change fractions.

The procedure described here, besides using a less costly grade of silica gel, appears to offer substantially better resolution than is claimed for the obvious alternative, flash chromatography. This is not a minor consideration, even for "one spot" reactions. We have routinely observed that samples purified as outlined here, followed by bulb-to-bulb distillation to remove traces of solvent residue, are satisfactory for elemental analysis.

Acknowledgment. This work was supported by the National Institutes of Health (Grant No. GM 15431). We are grateful to Professor W. C. Still for sharing his procedures with us.

Supplementary Material Available: Figures 1-3, with accompanying legends describing details of column preparation and operation (4 pages). Ordering information is given on any current masthead page.

New Highly Fluorescent Derivative of Adenosine.
Cyclization of Adenosine with 1'-Methylthiaminium Ion

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Received September 30, 1981

Considerable effort has been expended to convert adenosine (I, Chart I) into fluorescent derivatives. Such conversions not only provide an ultrasensitive method of detecting I but also furnish fluorophores which are useful bioprobes.}

Successful transformations largely include those which fuse a five-membered ring onto I by incorporating N-1 and the 6-amino group along with a reagent such as chloroacetaldehyde or glyoxal. Emphasis now is being placed on the synthesis of new fluorescent derivatives of heteroaromatic components of nucleic acids by annulation to give six-membered rings.

We report the preparation of a novel, highly fluorescent derivative of I. Two heterocyclic rings are fused onto I, both six membered, by treatment with 1'-methylthiaminium ion (II), a derivative of vitamin B1.

Results and Discussion

Compounds I and II readily react in refluxing methanol containing 2,4,6-trimethylpyridine catalyst. Proton and 13C NMR show that the product does not contain the thiazole ring (L) from II. In view of the many facile nucleophilic substitution reactions which II undergoes, I must be bonded to II at its CH2 group in place of the thiazole ring. Elemental analyses reveal that the substitution product is cyclic, cyclization proceeding by the loss of an amino group as ammonia. Therefore, the product is likely to have structure III or IV, both containing four fused heterocyclic rings having a total of seven annular nitrogen atoms.

Regioisomers III and IV differ by having the orientations of the two reactants reversed on cyclization. Isomer III has the CH2 group of II bonded to N-1 of I. One of the two amino groups is incorporated into the new ring, the other is lost as ammonia. Isomer IV has the CH2 chain attached to the 6-amino group of I; N-1 of I is bonded to position 4 of II in place of its amino substituent.

Differentiation between these two isomers was achieved by means of a nuclear Overhauser effect (NOE) involving