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# COMPOUND 30.4, AN UNUSUAL CHLORINATED 1,4-BENZOXAZIN-3-ONE DERIVATIVE FROM CORN (ZEA MAYS).

Ngo Le-Van* and Stephen J. Wratten

Monsanto Agricultural Products Company, 800 N. Lindbergh Blvd., St. Louis, Missouri 63167, USA

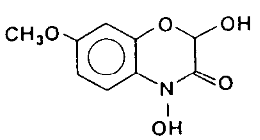
Summary: An unusual chlorinated 1,4-benzoxazin-3-one derivative has been isolated from young corn roots. Its structure was established by spectroscopic techniques ($^{1}$H-, $^{13}$C-NMR, EI and CI-MS, UV and IR) and by correlation with known benzoxazinone derivatives. The assignments of all protons could be made by $^{1}$H shift-correlated 2-D NMR techniques. The chlorinated benzoxazine derivative has been confirmed to be naturally occurring in a second carefully planned growth-chamber experiment.

Cyclic hydroxamic acids (e.g. DIMBOA, 1) are present in the cereal grasses, maize, wheat and rye and have been reported to be important in resistance of these plants to fungi and insects [1]. They are found in these plants mainly as glucosides, which are enzymatically transformed into the aglycone by the action of β-glucosidases upon crushing the plant tissue [2]. We now wish to report herein the structure elucidation of an unusual chlorinated benzoxazine glucoside, which we named compound 30.4, isolated from young corn roots. Compound 30.4 (2) was isolated from young corn roots which were grown in a green house for 12 days, together with the known benzoxazine glucosides 4, 6 [3, 4] and other unknown benzoxazine derivatives [5]. The pure compound could only be isolated and separated from other related derivatives by silica TLC after conversion to its respective peracetate. Compound 30.4-tetraacetate (3) has a very similar UV spectrum [$\lambda_{max}$ (Et$_2$O) 254 (1.059), 287 (0.433) nm and shoulder 298 nm] to that of 5. EI and CI-MS indicated a molecular ion at 559. (Found 559.1093, calculated for $C_{23}H_{26}NO_{13}Cl^{35}$ 559.1110)

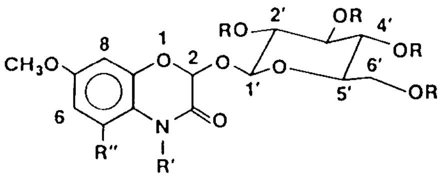
The presence of an unexpected chlorine atom in 3 was suggested by the M$^{+}$ + 2 in EI and CI-MS, and confirmed by the high resolution measurement of the mass fragments at m/z 559, 229, 211, 200 and 184 (Table 2). The IR spectrum of 3 dislayed bands at 1760, 1230 and 1720 cm$^{-1}$, characteristic of the carbonyl stretching vibration of acetate groups and the amide. 360 MHz $^{1}$H-NMR spectrum of 3 was similar to that of 5 and 7. It showed the presence of a methoxyl (δ3.809, Table 1) and acetate groups (δ2.068, 1.998, 1.989 and

1.929). In addition to the signals which are due to the amide and glucose protons (Table 1), the singlet at 5.692 and the doublets at δ6.688 (J = 2.7 Hz), 6.792 (J = 2.7 Hz) could only be assigned to H-2, and the meta aromatic protons H-6, H-8 (or vice versa). Biogenetic consideration and correlation of these $^1$H-NMR data with those of the acetylated products 5 and 7 of the known benzoxazine glucosides 4, [3] and 6 [4] suggest that the methoxy group in 3 is located at C-7, and hence the chlorine at C-5. Systematic double irradiation experiments led to the assignments of all protons of the sugar moiety, but not the two closely spaced aromatic protons. $^1$H shift-correlated 2-D NMR spectroscopy [6] was applied to unambiguously assign these protons. Figure 1 shows the contour plot of a COSY-90 experiment with N-type selection and fixed delay $\tau$ = 0.5 sec. The diagonal signals are present for every signal in the normal 1-D spectrum. The off-diagonal contour intensity arises from magnetization transfer through spin-spin relaxation of coupled spin systems. For a given pair of coupled spins the diagonal and off-diagonal signals form the four corners of a square. The methoxy signal is coupled to both aromatic protons, confirming that it must be located between these two. Long range coupling between H-2 and H-8 could be observed in this 2-D NMR experiment (Figure 1), and hence the assignments of H-8 (higher field) and H-6 (lower field) were possible.

The $^{13}$C-NMR data of 3 were consistent with the proposed structure. The CH and $CH_3/CH_2/CH$ subspectra of 3 were obtained by using the heteronuclear multipulse DEPT (= Distorsionless Enhancement by Polarization Transfer) technique, which provides significant improvement in S/N. Five $CH_3$ (δ20.51, 20.56, 20.59, 20.71 and 56.32), one $CH_2$ (δ62.83), eight CH (δ69.11, 71.86,



DIMBOA 1



2 = R = R' = H, R'' = Cl

3 = R = Ac, R' = H, R'' = Cl

4 = R = R' = R'' = H

5 = R = Ac, R' = R'' = H

6 = R = R'' = H, R' = $OCH_3$

7 = R = Ac, R' = $OCH_3$, R'' = H

73.04, 73.11, 97.67, 102.09, 104.61 and 110.14), and nine quartenary C (δ118.70, 120.78, 143.19, 156.97, 160.00, 169.66, 170.01, 170.29 and 170.80) could be observed. Treatment of 2 with β-glucosidase gave the aglucone and glucose, confirming the nature and stereochemistry of the sugar moiety. The chlorinated benzoxazine derivative has been confirmed to be naturally occurring in a second growth-chamber experiment, where all organochlorine sources were carefully excluded from the growth media. In addition, one week exposure of 5 to chloroform at room temperature produced no trace of 3. Halogenated derivatives are very rarely found in the terrestrial plants; chlorinated sesquiterpenes have been reported recently from Compositae [7].

The structure of compound 30.4 is therefore 2-(5-chloro-2-hydroxy-7-methoxy-2H-1,4-benzoxazin-3[4H]-one)-β-D-glucopyranoside.

TABLE 1: 360 MHz $^1$H — NMR DATA OF 3, 5, 7 in ACETONE-D6

| PROTON | 3 | 5 | 7 |
|---|---|---|---|
| H-2 | 5.692, s | 5.631, s | 5.804, s |
| H-5 | — | 6.952, d | 7.198, d |
| H-6 | 6.792, d | 6.637, dd | 6.756, dd |
| H-8 | 6.688, d | 6.677, d | 6.718, d |
| H-1' | 5.222, d | 5.204, d | 5.219, d |
| H-2' | 4.865, dd | 4.866, dd | 4.862, dd |
| H-3' | 5.306, dd | 5.305, dd | 5.317, dd |
| H-4' | 4.998, dd | 4.999, dd | 5.000, dd |
| H-5' | 4.074, ddd | 4.077, ddd | 4.078, ddd |
| H-6' | 4.251, dd | 4.264, dd | 4.266, dd |
| | 4.176, dd | 4.165, dd | 4.162, dd |
| OMe | 3.809, s | 3.768, s | 3.930, 3.802, s |
| OAc | 2.068, s | 2.060, s | 2.091, s |
| | 1.998, s | 1.999, s | 1.997, s (6H) |
| | 1.989, s | 1.984, s | |
| | 1.929, s | 1.928, s | 1.929, s |
| NH | 9.495, $s_{br}$ | 9.809, $s_{br}$ | — |

J in Hz = (5-6) = 8.6; (6-8) = 2.7; (1'-2') = 8.1; (2'-3') = 9.8; (3'-4') = 9.7
(4'-5') = 9.9; (5'-6') = 5.2; (5'-6') = 2.4; (6'-6') = 12.4

TABLE 2: HIGH RESOLUTION MASS FRAGMENTS OF COMPOUND 30.4-TETRAACETATE (3).

| M/Z | CALCULATED | FOUND | COMPOSITION |
|---|---|---|---|
| 559 | 559.1110 | 559.1093 | $C_{23}H_{26}NO_{13}Cl^{35}$ |
| 229 | 229.0161 | 229.0159 | $C_9H_8NO_4Cl^{35}$ |
| 211 | 211.0038 | 211.0036 | $C_9H_6NO_3Cl^{35}$ |
| 200 | 200.0117 | 200.0116 | $C_8H_7NO_3Cl^{35}$ |
| 184 | 184.0159 | 184.0157 | $C_8H_7NO_3Cl^{35}$ |

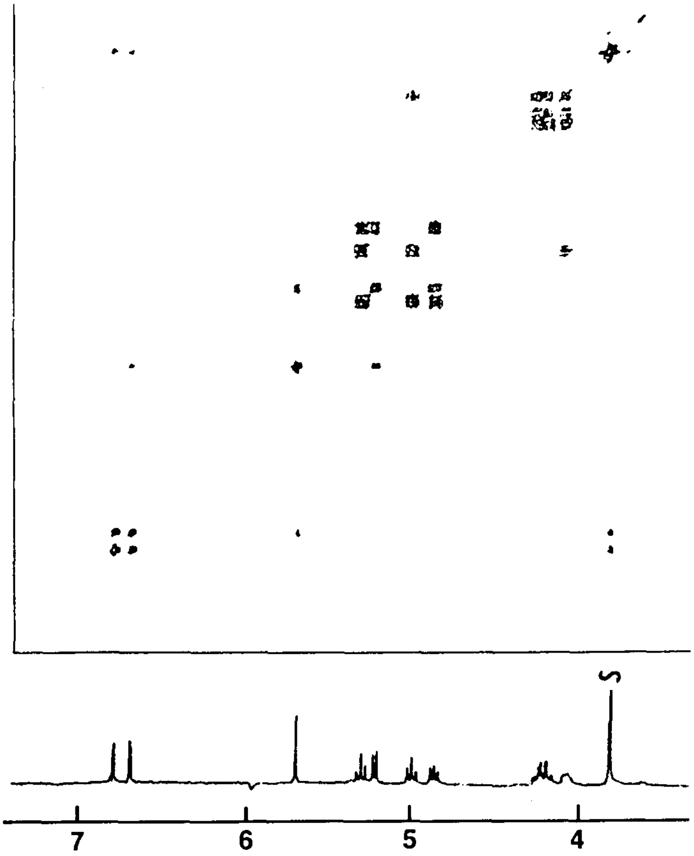


FIG. 1 — 360 MHZ $^1$H SHIFT — CORRELATED 2-D NMR OF COMPOUND 30.4 ACETATE IN ACETONE-D$_6$. CONTOUR PLOT OF A COSY-90 EXPERIMENT WITH N-TYPE SELECTION AND FIXED DELAY $\tau$ = 0.5 SEC.

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